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# Canadian Journal of Research

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VOL. 14, SEC. C. OCTOBER, 1936 NUMBER 10


## STUDIES ON THE INTERFERTILITY OF FOUR STRAINS OF *PUCCINIA HELIANTHI* SCHW.[1]


BY A. M. BROWN[2]



### Abstract

A distinct strain of *Puccinia Helianthi* was collected on each of the following four species, *Helianthus annuus*, *H. petiolaris*, *H. tuberosus*, and *H. subtuberosus*. All possible combinations of reciprocal crosses, haploid pustule with haploid pustule, and haploid pustule with diploid pustule, were made among these four strains. Interfertile strains, when crossed, give rise to aecia in the formerly haploid pustules. On this basis, the strains fell into two groups: A, the strains on *H. annuus* and *H. petiolaris*, and B, the strains on *H. tuberosus* and *H. subtuberosus*. The two strains in each group were highly interfertile; but the two strains of one group were highly intersterile with the two strains of the other group. A parallelism exists between the crossing behavior of certain varieties of *P. graminis* and that of these two groups. It is suggested that each of these two groups of *P. Helianthi* may represent a variety.


### Introduction

Studies on the crossing behavior of rust fungi have been confined to one or two cereal rusts. The present study deals with the sunflower rust (*Puccinia Helianthi* Schw.). This rust is known to be heterothallic (4) and to consist of different strains or races (2). An attempt was made to ascertain to what extent four strains of it, each collected on a different species of *Helianthus*, are interfertile.

From the studies that have been made on *Puccinia graminis* Pers., it seems that, within a variety such as *P. graminis Tritici* Erikss. & Henn., physiologic races (previously called forms) cross freely (9, 10, 11, 12, 14). The same seems to be true of races within the variety *P. rubigo-vera Tritici* Carl. (*P. triticina* Erikss.) (15). With varieties of *P. graminis* there seems to be some difference in the ease with which one variety will cross with another variety. The *Tritici* race apparently crosses rather freely with the *Secalis* race (11, 7), although the aeciospores from the *Secalis* pustules have difficulty in infecting either wheat or rye seedlings (7). Between the *Tritici* and *Agrostidis* (11, 7), and the *Tritici* and *Avenae* (8) varieties there is evidence of a rather high degree of intersterility. If degree of interfertility be taken as a criterion of the closeness of relationship between varieties, it would appear that the *Tritici* variety of *P. graminis* is more closely related to the *Secalis* variety than to either the *Agrostidis* or the *Avenae* varieties.


[1] *Manuscript received August 31, 1936.*
*Contribution No. 461 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa.*



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## Differentiation of Strains

Collections of telial material were made from *Helianthus annuus* L., *H. tuberosus* L., *H. petiolaris* Nutt., and *H. subtuberosus* Bourg.* From these collections uredial cultures were subsequently established on the respective hosts of origin. Uredospores from *H. annuus* were then used to inoculate the following species: *H. annuus*, *H. tuberosus*, *H. subtuberosus*, *H. petiolaris*, *H. subrhomboides* Rhyd., and *H. Maximiliani* Schrad. These species were likewise inoculated with uredospores from *H. tuberosus*, from *H. subtuberosus*, and from *H. petiolaris;* so that each species was inoculated with each of the four strains of rust. The results of these inoculations are given in Table I,

TABLE I

THE REACTION OF SIX SPECIES OF *Helianthus* TO COLLECTIONS OF *Puccinia Helianthi* OBTAINED FROM FOUR DIFFERENT SOURCES

| Source of rust | *H. annuus* | *H. tuberosus* | *H. subtuberosus* | *H. petiolaris* | *H. subrhomboides* | *H. Maximiliani* |
|---|---|---|---|---|---|---|
| *H. annuus* (Mammoth Russian) | S | O | O | O | O | O |
| *H. tuberosus* | S* | S | S | O | S* | O |
| *H. subtuberosus* | R | O | S | O | O | O |
| *H. petiolaris* | S | O | R | S | O | O |

* *Uredia on upper leaf surface only.*
S = *susceptible;* R = *resistant;* O = *immune.*

and they indicate that a distinct strain of rust was present on each of the four species, *H. tuberosus*, *H. annuus*, *H. subtuberosus*, and *H. petiolaris*. The host reactions were generally clearly defined, and no difficulty was experienced in distinguishing between strains. Plate I, A and B, shows the reaction of *H. annuus* to two of the strains.

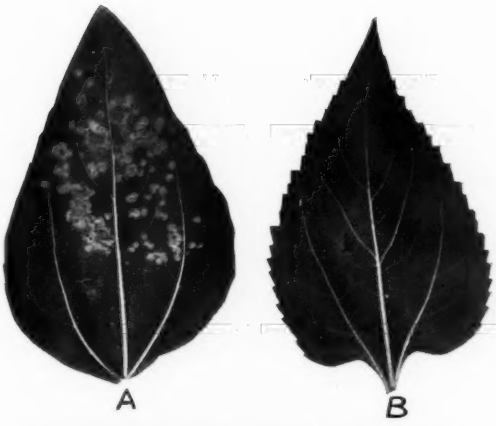
## Crossing Experiments

### A. By the Transfer of Pycnial Nectar

Telial material of each strain was produced in the greenhouse, and from this material haploid pustules of each strain were obtained on plants of Mammoth Russian sunflower, each plant being infected by a single strain. These pustules were made use of in the crossing experiments described below. The plants were protected from insects by screen-wire cages. To ensure reasonable certainty that the pustules used in the crosses were really haploid, only isolated pustules that were still haploid at the end of 21 days were chosen for the experiments.

* *Acknowledgment is made to Dr. R. C. Russell, Dominion Laboratory of Plant Pathology, Saskatoon, Sask., for providing material of H. petiolaris and H. subtuberosus.*

PLATE I



*A and B. Reactions of H. annuus to two strains of P. Helianthi. A. resistant reaction to the H. subtuberosus strain; B. susceptible reaction to the H. annuus strain. ×¼. C. Diploid mycelia of the annuus strain of P. Helianthi in contact with a haploid mycelium of the tuberosus strain. No aecia are produced.*

Following the method developed by Craigie (5), reciprocal crosses were made between all possible pairs of these four strains of rust. Briefly, the method was as follows. Pycnial nectar was collected from a number of haploid pustules of one strain to make a composite sample of nectar, and similar samples were collected from haploid pustules of each of the three strains. In making a cross between two strains, composite nectar of one strain was applied to the upper surface of pustules of the other strain, and nectar of the latter strain to pustules of the former. If, after a reciprocal cross was made between two strains, aecia arose within a few days in the pustules that received the exchanged nectar, the two strains were considered interfertile; if no aecia appeared, they were considered intersterile.

To serve as controls, haploid pustules of each strain were selfed, that is to say, composite nectar of a strain was applied to the upper surface of pustules of the same strain.

The data relative to the crosses made and the results obtained in this series of experiments are summarized in Table II. By way of explanation, it may be mentioned that, in this table, the arrow heads indicate the direction in

TABLE II

THE RESULTS OBTAINED FROM CROSSES MADE BETWEEN DIFFERENT STRAINS OF *P. Helianthi* ORIGINATING ON FOUR DIFFERENT SPECIES OF *Helianthus*

| Crosses | Number of pustules with aecia |
|---|---|
| *H. subtuberosus* strain (23) ⇆ *H. petiolaris* strain (34) | 0 |
| *H. subtuberosus* strain (11) ⇆ *H. tuberosus* strain (10) | 19 |
| *H. subtuberosus* strain (29) ⇆ *H. annuus* strain (61) | 0 |
| *H. tuberosus* strain (22) ⇆ *H. petiolaris* strain (45) | 0 |
| *H. tuberosus* strain (62) ⇆ *H. annuus* strain (71) | 0 |
| *H. petiolaris* strain (16) ⇆ *H. annuus* strain (29) | 42 |
| *H. annuus* strain, selfed (44) | 40 |
| *H. tuberosus* strain, selfed (23) | 21 |
| *H. subtuberosus* strain, selfed (20) | 19 |
| *H. petiolaris* strain, selfed (26) | 24 |

which the pycnial nectar was transferred, and the figures enclosed by brackets give the number of pustules that received the transferred nectar. For example, in the first reciprocal cross, composite nectar of the *H. subtuberosus* strain was transferred to 34 haploid pustules of the *H. petiolaris* strain, and composite nectar of the *H. petiolaris* strain to 23 pustules of the *H. subtuberosus* strain.

Although the number of pustules used in the crosses is relatively small, the results given in Table II indicate that there is at least a high degree of intersterility between the strain of *P. Helianthi* on *H. subtuberosus* and the one on *H. petiolaris* or the one on *H. annuus*, but a high degree of interfertility between the strain on *H. subtuberosus* and the one on *H. tuberosus*. The strain on *H. annuus* and the one on *H. petiolaris* are highly interfertile, but both are highly intersterile with the strain on *H. tuberosus*.

Additional tests were made with the *annuus* and *tuberosus* strains, each strain being cultured on its respective host. Of the two strains a total of 43 haploid pustules were crossed, but none produced aecia. Fifteen haploid pustules of the former strain and 12 of the latter were selfed. Aecia formed in 12 and 9 of the pustules, respectively.

### B. By the Diploidisation Method

The validity of these results was tested by a second method of crossing the strains. It has been shown that, in the sunflower rust (3), a diploid mycelium will, when it interacts with a haploid mycelium, diploidise it and initiate the formation of aecia. This result was obtained with rust collected on the cultivated sunflower.

In the second series of experiments, haploid pustules of the four strains of rust were obtained on plants of Mammoth Russian sunflower. Each plant bore pustules of a single strain. The haploid pustules used in these tests were isolated on the leaves, and all were at least 21 days old. The method of making a cross was as follows. A leaf bearing a haploid pustule of one strain was inoculated near the periphery of the pustule with uredospores (diploid) of another strain. Uredia arose in close proximity to the haploid pustule and thus an opportunity was afforded for the interaction of the two types of mycelia. All possible combinations of haploid with diploid mycelia were made among the four strains of rust. If, after reciprocal pairing of haploid and diploid pustules of two strains, aecia were produced in the formerly haploid pustules, the two strains were considered interfertile, if no aecia appeared, intersterile (Plate I, C).

As a control for each cross, both parental strains were "selfed", that is to say, haploid and diploid pustules of the same strain were brought together, after the manner of making a cross described above.

The results of this series of experiments are summarized in Table III. With the exception of a slight deviation in the cross between the *annuus* and the

TABLE III

The results of pairing haploid with diploid mycelia in four strains of *P. Helianthi*

| Haploid strain | Diploid strain | Number of haploid pustules | Number of pustules with aecia |
|---|---|---|---|
| *H. annuus* | *H. subtuberosus* | 20 | 2 |
| *H. subtuberosus* | *H. annuus* | 26 | 1 |
| *H. annuus* | *H. tuberosus* | 23 | 0 |
| *H. tuberosus* | *H. annuus* | 74 | 0 |
| *H. petiolaris* | *H. subtuberosus* | 18 | 0 |
| *H. subtuberosus* | *H. petiolaris* | 7 | 0 |
| *H. tuberosus* | *H. petiolaris* | 6 | 0 |
| *H. petiolaris* | *H. tuberosus* | 10 | 0 |
| *H. annuus* | *H. petiolaris* | 34 | 31 |
| *H. subtuberosus* | *H. tuberosus* | 26 | 22 |
| *H. annuus* | *H. annuus* | 10 | 10 |
| *H. petiolaris* | *H. petiolaris* | 27 | 24 |
| *H. subtuberosus* | *H. subtuberosus* | 19 | 18 |
| *H. tuberosus* | *H. tuberosus* | 31 | 29 |

*subtuberosus* strains, the results of these experiments are in agreement with those of the first series. In the first series the *annuus* and the *subtuberosus* strains appeared to be perfectly intersterile, but, as shown in Table III, a slight degree of interfertility is indicated. Out of 46 pairings of haploid and diploid pustules, three of the haploid pustules produced aecia.

In these three pustules, the aecia were few in number and these few arose at the margins of the haploid pustules, just where they made contact with diploid pustules. The aecia were more or less rudimentary in appearance and viable aeciospores were recovered from only some of them. When two interfertile strains unite, aecia usually appear first near the point of contact between the haploid and the diploid pustule and then continue to appear progressively across the formerly haploid pustule until the whole undersurface is studded with aecia.

Aeciospores from these three pustules were used to inoculate Mammoth Russian plants. It was only on some plants that had been left in moist chambers for eight days that infections were secured. These infections were sparse. Apparently the aeciospores were but weakly viable. Uredospores produced by these infections were likewise subnormal with respect to viability.

A striking feature of the germination of these uredospores was the abnormal type of germ tube produced. Many of the germ tubes resembled the promycelia formed by teliospores of *P. Helianthi* germinated in hanging drops of water. Most of the germ-tube tips were swollen. Apical swelling in uredospore germ tubes is apparently not an uncommon phenomenon, for it was observed as early as 1854 by Tulasne (13) and later by other investigators. Ezekiel (6) found it common in physiologic races (forms) of *P. graminis Tritici*, but much more abundant in some races than in others. In the present case, however, it was observed only in the germ tubes of uredospores that were derived from the cross between the nearly intersterile *annuus* and *subtuberosus* strains of rust.

## Discussion

The identity of the four strains of rust has not been determined on all the differentials used by Bailey (2). Only three of his differential species were available, namely, *H. annuus* (Mammoth Russian), *H. tuberosus* (probably his second strain of this species), and *H. Maximiliani*. There is no surety, however, that these representatives were genotypically identical, respectively, with the three that he worked with. The likelihood is that they were not; for, as he pointed out, sunflowers are practically self-sterile, so that each species must include a large number of genotypes, and, therefore, "the reactions indicated for the particular collection of the species worked with cannot be expected to hold for all samples of the same species". However, the strain collected on *H. annuus* produced the same types of infection on the three differentials of his mentioned above and is probably identical with his race (form) 1. The other three strains lacked agreement with both of his other races.

It has been shown in the present study that, on a basis of their interfertility, the four strains fall into two distinct groups, which, for convenience, may be called A and B. Group A consists of the two strains on *H. annuus* and *H. petiolaris*, and Group B of the two strains on *H. tuberosus* and *H. subtuberosus*. Arthur (1) concluded from his own work and that of others that, within the species *P. Helianthi*, "a number of physiologic forms exist, but none so distinctive that they could be called varieties". However, the similarity of the crossing behavior of the two strains in either Group A or Group B with that of races within a variety of *P. graminis*, and the similarity of the crossing behavior of the two groups, A and B, with that of the variety *Tritici* and the variety *Agrostidis* or *Avenae* of *P. graminis* suggest strongly that each group represents a variety.

To this concept there is the objection that the parallelism does not extend to the hosts. The varieties of *P. graminis* are more or less specialized to different genera, *e.g.*, the *Tritici* variety to wheat, the *Avenae* variety to oats, etc., whereas the two groups of *P. Helianthi* occur on species of the same genus. Moreover, one host, Mammoth Russian, is susceptible to the two strains of Group A, and to one strain (on *H. tuberosus*) of Group B. This statement, perhaps, requires some qualification. What actually happens is that, on Mammoth Russian, the strain collected on *H. tuberosus* produces a susceptible type of reaction on the upper surface of the leaves, but no uredia are formed on the lower surface. In susceptible species, uredia regularly form on the lower surface of the leaves. If the absence of uredia on the lower surface indicates resistance, Mammoth Russian can be considered resistant to both strains of Group B, in which case the parallelism is brought somewhat more closely into line.

As has been mentioned above, there seems to be considerable difference in the interfertility of different varieties of *P. graminis*. The crossing behavior of the two groups of *P. Helianthi* seems to resemble that of the *Tritici* variety and the *Agrostidis* or *Avenae* variety of *P. graminis*. Before drawing any definite conclusion as to whether these two groups of *P. Helianthi* represent different varieties, it would be well to await the results of crossing experiments with varieties of other rust fungi, such as varieties of *Puccinia coronata* Cda. or of *P. rubigo-vera* Carl. It is not, therefore, proposed here to raise the two groups of *P. Helianthi* to the rank of variety, and burden each with a trinomial designation, although further studies may show this to be necessary. For the time being, the data presented are given for what they are worth as a contribution to the point under discussion.

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# INHERITANCE STUDIES OF SEVERAL QUALITATIVE AND QUANTITATIVE CHARACTERS IN SPRING WHEAT CROSSES BETWEEN VARIETIES RELATIVELY SUSCEPTIBLE AND RESISTANT TO DROUGHT[1]

By J. H. Torrie[2]

## Abstract

Genetic studies were made in the $F_2$ and $F_3$ generations of the crosses, Selection I-28-60 × Milturum, Reward × Caesium, and Caesium × Marquis. It was found that the character glume color was controlled by either one or two factor pairs in the cross Selection I-28-60 × Milturum, and by two factor pairs in the crosses Reward × Caesium and Caesium × Marquis. The characters, awning, straw color, glume pubescence and spike regularity were each governed by one factor pair, while three factor pairs were operative in the inheritance of seed color.

Polymeric factors apparently control the inheritance of the quantitative characters straw strength, plant height, earliness and grain yield. A partial dominance of strong straw and earliness was found in the crosses Reward × Caesium and Caesium × Marquis. Tallness and low grain yield were partially dominant in the cross Reward × Caesium. Evidence for transgressive segregation of earliness was obtained in the cross between Caesium and Marquis.

The characters glume color, awning, straw color, glume pubescence and spike regularity were inherited independently. White straw color and earliness were definitely associated in the crosses Reward × Caesium and Caesium × Marquis. The characters straw color and plant height were loosely linked in the Caesium × Marquis cross. Grain yield was not significantly correlated with straw strength, plant height or earliness in the cross between Reward and Caesium. Small but significant relationships were found among the characters straw strength, plant height and earliness in the crosses Reward × Caesium and Caesium × Marquis. The relation between heading and maturity was studied only in the $F_4$ of Reward × Caesium, in which case a strong positive correlation was obtained.

## Introduction

Drought is one of the major limiting factors in successful wheat production in most areas of western Canada. This has been particularly exemplified during the past five years. The development of a wheat possessing a measure of drought resistance would be a forward step in stabilizing wheat production. With this object in view, the University of Alberta, in 1929, in co-operation with the National Research Council of Canada, initiated a program of breeding drought resistant wheat (1, 2). The studies reported herein, on the genetics of several qualitative and quantitative characters, were undertaken as part of this program. Studies on the drought resistance (5) and quality (4) of these hybrids are being reported in separate papers.

[1] *Manuscript received September 3, 1936.*

*Contribution from the Department of Field Crops, University of Alberta, Edmonton, Canada, with financial assistance from the National Research Council of Canada. This work is part of a thesis presented to the Committee on Graduate Studies, University of Alberta, in partial fulfilment of the requirements for the degree of Master of Science. Published as Paper No. 101 of the Associate Committee on Grain Research of the National Research Council and the Dominion Department of Agriculture.*

[2] *Formerly Research Assistant in Genetics and Plant Breeding, University of Alberta; now Graduate Assistant, Department of Agronomy, University of Wisconsin, Madison, Wisconsin.*

## Materials and Experimental Methods

The material used for these studies consisted of the $F_2$ and $F_3$ generations of the crosses, Selection I-28-60 × Milturum, Reward × Caesium and Caesium × Marquis, and certain $F_4$ lines of Reward × Caesium. Reciprocal crosses were made only between the varieties Caesium and Marquis. Since no significant differences for any of the characters studied were obtained in the reciprocal crosses, the data were treated collectively. Table I gives a comparison of the parental varieties for the characters studied.

TABLE I

CHARACTERS OF PARENTAL VARIETIES STUDIED

| Characters studied | Variety | | | | |
|---|---|---|---|---|---|
| | Caesium 0.111 | Milturum 0.321 | Selection I-28-60 | Marquis | Reward |
| C.A.N.* | 1256 | 1415 | — | 1621 | 1509 |
| Glume color | Brown | Brown | White | White | White |
| Glume pubescence | Glabrous | Glabrous | Glabrous | Glabrous | Pubescence |
| Awning | Awned | Awnletted | Awnletted | Awnletted | Awnletted |
| Spike regularity | Irregular | Regular | Regular | Regular | Irregular |
| Straw color | Purple | Purple | White | White | White |
| Seed color | Red | Red | Red | Red | Red |
| Strength of straw | Weak | Weak | Strong | Strong | Strong |
| Height of plant | Tall | Tall | Short | Medium | Short |
| Maturity | Late | Late | Medium | Medium | Early |

**C.A.N. Canadian Accession Number.*

Caesium 0.111 and Milturum 0.321 are red spring wheats obtained, in 1928, from Dr. Talanoff of the West Siberian Plant Breeding Station at Omsk, U.S.S.R. Talanoff (29) states that under conditions of extreme drought these two wheats proved to be the most productive and drought resistant of all the varieties tested. Selection I-28-60, Marquillo × (Marquis-Kanred) is a hard red spring wheat, selected at the University of Alberta from a strain introduced in 1928 from the University of Minnesota. Marquis and Reward are hard red spring wheats which originated at the Central Experimental Farm, Ottawa.

The $F_2$ lines of all crosses and the $F_3$ lines of Selection I-28-60 × Milturum were planted in five-foot rows, approximately 25 seeds being planted in each row. The $F_3$ and $F_4$ lines of Reward × Caesium and the $F_3$ lines of Caesium × Marquis were planted in single ten-foot rows, approximately 50 seeds being planted in each row. Parental varieties were sown at intervals of approximately 30 rows.

The characters studied were placed in two groups; qualitative and quantitative. Those treated qualitatively were classified into definite categories. The qualitative characters are glume color, awning, straw color, seed color, glume pubescence and spike regularity. The quantitative characters which were classified by arbitrary numerical values are straw strength, plant height, earliness and grain yield.

The methods of handling and classifying the material, for any individual character, are presented later with the discussion of that character. Data relative to $F_2$ populations derived from different $F_1$ plants were computed separately. These were combined after it was ascertained that there were no significant differences between the several $F_2$ populations. The methods described by Fisher (14) were used in the analysis of the data.

## Inheritance of Qualitative Characters

Table II shows the breeding behavior of glume color in the cross Selection I-28-60 × Milturum. The breeding behavior of the Reward × Caesium cross for the characters, glume color, awning, straw color, seed color and glume pubescence is given in Table III. Table IV presents the breeding behavior of Caesium × Marquis for the characters, glume color, awning, straw color, seed color and spike regularity. In these three tables the data obtained are given for each character for the $F_2$ segregation based on their $F_3$ breeding behavior. These are followed by the data for the segregation of the plants in the $F_3$ from heterzygous $F_2$ plants.

TABLE II

THE BREEDING BEHAVIOR OF SELECTION I-28-60 × MILTURUM CROSS FOR GLUME COLOR AND TESTS OF GOODNESS OF FIT

| Number of $F_3$ lines or plants | Expected ratio | Observed | Calculated | $\chi^2$ | P lies between |
|---|---|---|---|---|---|
| 170 lines | 1 : 2 : 1 | 40 : 88 : 42 | 42.5 : 85.0 : 42.5 | 0.27 | .90 and .80 |
| 1711 plants | 3 : 1 | 1269 : 442 | 1283.3 : 427.8 | 0.64 | .50 and .30 |
| 167 lines | 7 : 4 : 4 : 1 | 86 : 26 : 43 : 12 | 73.1 : 41.8 : 41.8 : 10.4 | 8.50 | .05 and .02 |
| 710 plants | 15 : 1 | 660 : 50 | 665.6 : 44.4 | 0.76 | .50 and .30 |
| 1261 plants | 3 : 1 | 936 : 325 | 947.8 : 315.2 | 0.40 | .70 and .50 |

*Glume Color*

In several crosses of *T. vulgare* wheats, Biffen (7) found that the difference between brown and white glume color is monogenic, the brown glume color being dominant. In crosses made between some white-chaffed and brown-chaffed Swedish "land wheats", Nilsson-Ehle (24) obtained in the $F_2$ a ratio of 3 brown : 1 white; or in other cases 15 brown : 1 white.

In the present investigation the inheritance of glume color was studied in all three crosses. Variation was noticed in the pigmentation of the brown glume. Since this was believed to be influenced considerably by environmental factors, the plants were classified into two general classes, brown and white. No difficulty was experienced in distinguishing the plants falling into these two categories.

Eight $F_2$ populations from the Selection I-28-60 × Milturum cross were monogenic, while seven were dihybrids for the expression of glume color. Table II gives the observed and calculated ratios. Satisfactory fits to the theoretical ratios were obtained in all cases save for the $F_2$ populations

segregating into the dihybrid ratio 7 : 4 : 4 : 1. A P value lying between .05 and .02 was obtained in this instance. The poor fit is largely due to an excess of homozygous brown lines and a corresponding deficiency of lines segregating into the 15 : 1 ratio. This is explainable on the assumption that the number of plants in each $F_3$ line (20–25) was too few to obtain segregation in all the $F_3$ lines heterozygous for two-factor pairs.

In the crosses Reward × Caesium and Caesium × Marquis, two independent factor pairs were found to be operative in the inheritance of glume color. The experimental data are given in Tables III and IV.

*Awning*

The literature on the inheritance of awning in wheat has been recently reviewed by Kilduff (23). In crosses between awnletted and fully awned wheats, a single-factor-pair difference has been found by practically all investigators. In a cross Sonora × Reliance, Clark and Quisenberry (13) obtained in the $F_3$ too few awnletted lines and an excess of segregating lines. The segregating lines had a great excess of awned plants which, the authors suggest, may be the result of the presence of a dominant factor-pair for the awned condition. In a Kota × Garnet cross, Kilduff (23) found a very poor fit for the one-factor-pair hypothesis. He obtained an excess of true breeding fully awned lines, which he believes is due to more than one factor pair operating in this cross.

Awning was studied in the crosses Reward × Caesium and Caesium × Marquis. The awnletted class had a considerable range in its degree of expression. The difference, however, between the awnletted and awned plants was clear cut. The difference between the fully awned condition of Caesium and the awnletted condition of Reward and Marquis was found to be monogenic. This is shown by the data in Tables III and IV. A poor fit to the theoretical ratio was obtained in the $F_3$ segregating lines of Caesium × Marquis. The poor agreement was caused by excess of awned plants. No satisfactory genetic explanation can be offered to account for this discrepancy.

*Straw Color*

An examination of the available literature of inheritance in wheat revealed no instance reporting the inheritance of straw color. This character was studied in the crosses of Caesium with Reward and Marquis. On account of a marked bleaching of the purple color, some difficulty was experienced in the $F_2$ classification of the culm color for certain plants. In this respect very little difficulty occurred in the $F_3$. The upper leaf sheath was removed from the plants of which the culm color was doubtful. This resulted in the uncovering of an unbleached portion of the culm. No great difficulty was experienced in distinguishing between bleached purple and white culms. In both crosses, as shown by the data in Tables III and IV, the difference between purple and white straw color is dependent upon one factor pair. Purple straw color was partially dominant.

TABLE III

THE BREEDING BEHAVIOR OF REWARD × CAESIUM CROSS FOR SEVERAL QUALITATIVE CHARACTERS, AND TESTS OF GOODNESS OF FIT

| Character | Number of $F_3$ lines or plants | Expected ratio | Observed | Calculated | $\chi^2$ | P lies between |
|---|---|---|---|---|---|---|
| Glume color | 132 lines | 7 : 4 : 4 : 1 | 53 : 28 : 38 : 13 | 57.8 : 33 : 33 : 8.2 | 4.68 | .20 and .10 |
| Glume color | 1059 plants | 15 : 1 | 988 : 71 | 992.8 : 66.2 | 0.37 | .70 and .50 |
| Glume color | 1612 plants | 3 : 1 | 1213 : 399 | 1209 : 403 | 0.53 | .90 and .80 |
| Awning | 132 lines | 1 : 2 : 1 | 29 : 71 : 32 | 33 : 66 : 33 | 0.89 | .70 and .50 |
| Awning | 2886 plants | 3 : 1 | 2129 : 757 | 2164.5 : 721.5 | 2.31 | .20 and .10 |
| Straw color | 132 lines | 1 : 2 : 1 | 28 : 77 : 27 | 33 : 66 : 33 | 3.68 | .20 and .10 |
| Straw color | 3175 plants | 3 : 1 | 2401 : 774 | 2381.2 : 793.8 | 0.65 | .50 and .30 |
| Seed color | 132 lines | 37 : 8 : 12 : 6 : 1 | 89 : 8 : 21 : 11 : 3 | 76.3 : 16.5 : 24.7 : 12.4 : 2.1 | 7.55 | .20 and .10 |
| Seed color | 341 plants | 63 : 1 | 333 : 8 | 335.7 : 5.3 | 1.34 | .30 and .20 |
| Seed color | 835 plants | 15 : 1 | 780 : 55 | 782.8 : 52.2 | 0.16 | .70 and .50 |
| Seed color | 452 plants | 3 : 1 | 341 : 11 | 339 : 113 | 0.04 | .90 and .80 |
| Glume pubescence | 132 lines | 1 : 2 : 1 | 31 : 64 : 37 | 33 : 66 : 33 | 0.94 | .70 and .50 |
| Glume pubescence | 2557 plants | 3 : 1 | 1808 : 749 | 1917.7 : 639.3 | 25.15 | .01 and .00 |

TABLE IV

THE BREEDING BEHAVIOR OF CAESIUM × MARQUIS CROSS FOR SEVERAL QUALITATIVE CHARACTERS, AND TESTS OF GOODNESS OF FIT

| Character | Number of $F_3$ lines or plants | Expected ratio | Observed | Calculated | $\chi^2$ | P lies between |
|---|---|---|---|---|---|---|
| Glume color | 275 lines | 7 : 4 : 4 : 1 | 116 : 68 : 66 : 25 | 120.3 : 68.8 : 68.8 : 17.2 | 3.90 | .30 and .20 |
| Glume color | 2671 plants | 15 : 1 | 2471 : 200 | 2504.1 : 176.9 | 1.06 | .30 and .20 |
| Glume color | 2730 plants | 3 : 1 | 2075 : 655 | 2047.5 : 682.5 | 1.48 | .30 and .20 |
| Awning | 296 lines | 1 : 2 : 1 | 79 : 131 : 86 | 74 : 148 : 74 | 3.24 | .20 and .10 |
| Awning | 5328 plants | 3 : 1 | 3782 : 1546 | 3996 : 1332 | 41.08 | .01 and .00 |
| Straw color | 296 lines | 1 : 2 : 1 | 83 : 137 : 76 | 74 : 148 : 74 | 1.96 | .50 and .30 |
| Straw color | 5714 plants | 3 : 1 | 4317 : 1397 | 4285.5 : 1428.5 | 0.92 | .50 and .30 |
| Seed color | 274 lines | 37 : 8 : 12 : 6 : 1 | 185 : 23 : 37 : 22 : 7 | 158.4 : 34.2 : 51.4 : 25.7 : 4.3 | 14.47 | .01 and .00 |
| Seed color | 939 plants | 63 : 1 | 916 : 23 | 924.3 : 14.7 | 4.81 | .05 and .02 |
| Seed color | 1453 plants | 15 : 1 | 1355 : 98 | 1352.2 : 90.8 | 0.57 | .50 and .30 |
| Seed color | 887 plants | 3 : 1 | 686 : 201 | 665.3 : 221.7 | 2.59 | .20 and .10 |
| Spike regularity | 296 lines | 1 : 2 : 1 | 57 : 172 : 67 | 74 : 148 : 74 | 8.46 | .02 and .01 |
| Spike regularity | 6895 plants | 3 : 1 | 5027 : 1868 | 5172 : 1724 | 16.00 | .01 and .00 |

*Seed Color*

Kilduff (23) has recently reviewed the literature on the inheritance of seed color in wheat. Previous investigators have shown that one, two or three factor pairs govern the inheritance of this character.

The inheritance of seed color was studied in crosses of the variety Caesium with Reward and Marquis. Three factor pairs were found to govern the expression of seed color in these crosses. The observed and calculated numbers are given in Tables III and IV. In the $F_3$ of Caesium × Marquis a P value less than .01 was obtained. The population of an $F_3$ line (45–50) plants was too small to obtain segregation in all the lines heterozygous for the tri-hybrid ratio.

In the cross Caesium × Marquis, two $F_2$ populations with more than 1000 $F_3$ plants, gave no white seeded segregates. In this case one of the factor pairs for red seed color of Marquis and that of Caesium are allelomorphic.

*Glume Pubescence*

Biffen (7) found that the difference between glabrous and pubescent glumes was monogenic in a pubescent *T. turgidum* × glabrous *T. vulgare* and also in an intra *T. vulgare* cross. In several crosses of pubescent *T. durum* × glabrous *T. vulgare* and pubescent *T. vulgare* and glabrous *T. vulgare*, Howard and Howard (22), obtained an intermediate condition in the $F_1$ and a ratio of 15 pubescent : 1 glabrous in the $F_2$.

A one factor pair difference was found between pubescent and glabrous glumes in the cross Reward × Caesium. The data are given in Table III. A P value less than .01 was obtained in the segregating $F_3$ lines. This poor agreement is largely on account of the excess of glabrous plants, and a corresponding deficiency of pubescent ones. A considerable range was noticed in the degree of glume pubescence. This was probably due to the intermediate heterozygous condition, which made it difficult to distinguish between slightly pubescent and glabrous plants. It is also possible that the pubescence of a number of slightly pubescent plants may have been brushed off in handling, thus making it difficult to distinguish them from glabrous plants. For these reasons it is believed that the excess of glabrous plants resulted from the classification of certain pubescent plants as glabrous.

*Spike Regularity*

An examination of the available literature of the inheritance in wheat revealed no case reporting the inheritance of spike regularity.

For the character of spike regularity, the plants were classified into two categories, regular and irregular. The regular spike refers to the typical spike arrangement found in most common wheat varieties, in which the spikelets are arranged more or less parallel to the rachis from a lateral view, thus giving the spike a uniform appearance or regular outline. The irregular spike condition refers to the arrangement found in Reward and Caesium,

in which case most of the spikelets are arranged at a distinct angle to the rachis, thus giving an unsymmetrical or irregular appearance to the spike. These are shown in Fig. 1.

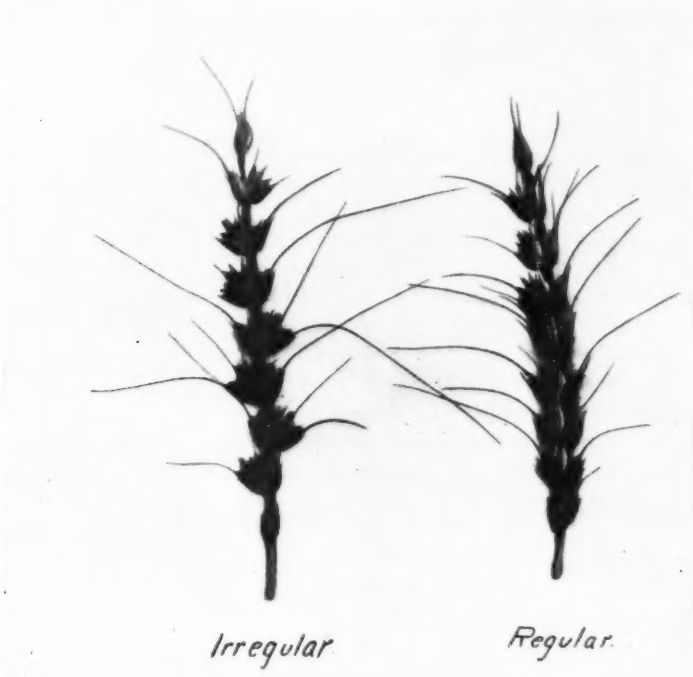


FIG. 1. *Irregular and regular spike.*

Table IV shows the breeding behavior of this character in the cross Caesium × Marquis. Although poor fits were obtained for the one factor pair hypothesis, it is quite evident that no other simple genetic scheme would fit the data. Some difficulty was experienced in the classification of certain plants, owing to an intermediate expression of this character. The intermediate condition resulted from a partial dominance of the irregular spike.

## Inheritance of Quantitative Characters

*Strength of Straw*

The ability of wheat to resist lodging is a very important agronomic character. Strength of straw is influenced considerably by environmental factors which render a genetical study very difficult. Kilduff (23) has recently reviewed the literature on the inheritance of this character. Harrington (20) and Kilduff (23) both suggest that several factor pairs govern the inheritance of straw strength.

Strength of straw was studied in the $F_3$ of Caesium crossed with Reward and Marquis and in certain $F_4$ lines of Reward × Caesium. A satisfactory differentiation for straw strength was secured for all the material studied. An estimation of straw strength was obtained for each line by the lodging index. This index was calculated by the formula:

$$\frac{\%\ \textit{plants lodged} \times \textit{average angle off the vertical}}{90}$$

*Earliness of Heading*

Biffen (7) and Florell (15), both report that one factor pair governs the inheritance of earliness in wheat. Florell (15) suggests, however, the possible presence of a number of minor modifying factor pairs. Nilsson-Ehle (25), Thompson (30), Harrington (20), Stephens (28) and Florell (16), all suggest that two or more factor pairs are present. Transgressive segregation for earliness was found by Thompson (30), Bryan and Pressley (9), Harrington (20) and Clark and Hooker (12). Fruwirth, cited by Florell (15), Florell (15), Clark (10) and Stephens (28) report the dominance or partial dominance of earliness. On the other hand, Freeman (17) and Bryan and Pressley (9) found a dominance or partial dominance of lateness.

Environmental as well as genetical factors play an important role in determining the growth period of wheat varieties. Date of heading is less influenced by environmental factors than is date of maturity in any given season. For this reason, it is a better indication of earliness, in genetical studies, than is date of maturity. The emergence of the first spike from the sheath was used as the index of heading. Earliness was studied in the $F_3$ of Caesium crossed with Reward and Marquis and in selected $F_4$ lines of Reward × Caesium. During the heading period, for all the material studied, the weather was ideal, no heavy rains occurring to prevent the taking of daily notes. In the $F_3$ of Reward × Caesium, in half of the population the plants were tagged daily as they headed, while in the remainder of the population the total number of plants headed for each line was counted each day. In the $F_3$ of the cross Caesium × Marquis and the $F_4$ of Reward × Caesium the dates when the first plant headed, approximately 50% of the plants, and the last plant headed, were recorded for each line. The two methods were found to check very closely when applied to the $F_3$ lines of the cross between Reward and Caesium.

The distribution, for days from seedling emergence to heading, of the means for the $F_3$ and parental lines of Caesium crossed with Reward and Marquis are given in Table VII. The difference between Reward and Caesium of $9.3 \pm 0.25$ days and between Caesium and Marquis of $3.2 \pm 0.22$ days are both very significant. The distribution of the means of the $F_3$ lines in Reward × Caesium approaches a normal curve with a slight tendency to mass towards the mean of the early parent. Further studies in the $F_4$ of this cross showed that apparent homozygous lines occurred at several points intermediate between the means of the two parental varieties.

TABLE V

FREQUENCY DISTRIBUTION, MEAN AND STANDARD DEVIATION FOR LODGING INDEX OF REWARD × CAESIUM AND CAESIUM × MARQUIS $F_3$ AND PARENTAL LINES

| Parent or cross | Lodging index in 5 per cent classes | | | | | | | | | | | | Total number | Mean | Standard deviation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 8 | 13 | 18 | 23 | 28 | 33 | 38 | 43 | 48 | 53 | | | |
| Reward | 3 | 9 | – | – | – | – | – | – | – | – | – | – | 12 | 2.3±0.37 | 1.3±0.27 |
| Caesium | – | – | – | – | – | – | – | 2 | 6 | 4 | – | – | 12 | 38.9±0.65 | 2.3±0.47 |
| Reward × Caesium | 11 | 42 | 24 | 17 | 14 | 7 | 6 | 2 | 5 | 4 | – | – | 132 | 11.6±1.00 | 11.4±0.70 |
| Marquis | 7 | 20 | 2 | 1 | – | – | – | – | – | – | – | – | 30 | 3.0±0.49 | 2.7±0.34 |
| Caesium | – | – | – | – | – | – | – | 5 | 5 | 8 | 5 | 7 | 30 | 43.7±1.28 | 7.0±0.89 |
| Caesium × Marquis | 30 | 154 | 240 | 207 | 224 | 169 | 100 | 116 | 60 | 27 | 41 | 26 | 1394 | 18.9±0.37 | 13.7±0.25 |

TABLE VI

FREQUENCY DISTRIBUTION, MEAN AND STANDARD DEVIATION FOR HEIGHT OF PLANT OF REWARD × CAESIUM AND CAESIUM × MARQUIS $F_3$ AND PARENTAL LINES

| Parent or cross | Plant height in 3 cm. classes | | | | | | | | | | | | Total number | Mean | Standard deviation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 104 | 107 | 110 | 113 | 116 | 119 | 122 | 125 | 128 | 131 | 134 | 137 | | | |
| Reward | – | – | 3 | 8 | 1 | – | – | – | – | – | – | – | 12 | 112.5±0.49 | 1.7±0.24 |
| Caesium | – | – | – | – | – | – | – | – | 1 | 9 | 2 | – | 12 | 131.2±0.43 | 1.5±0.31 |
| Reward × Caesium | 1 | 1 | – | 1 | 4 | 12 | 32 | 26 | 26 | 16 | 11 | 2 | 132 | 125.3±0.48 | 5.4±0.34 |
| Marquis | – | – | 1 | 3 | 6 | – | – | – | – | – | – | – | 10 | 114.5±0.64 | 2.0±0.45 |
| Caesium | – | – | – | – | – | – | – | 1 | – | 4 | 3 | 2 | 10 | 132.5±1.07 | 3.4±0.76 |
| Caesium × Marquis | – | 1 | 2 | 8 | 21 | 44 | 67 | 70 | 56 | 18 | 8 | 2 | 297 | 123.6±0.28 | 5.0±0.21 |

TABLE VII

FREQUENCY DISTRIBUTION, MEAN AND STANDARD DEVIATION FOR DAYS FROM SEEDLING EMERGENCE TO HEADING OF REWARD × CAESIUM AND MARQUIS × CAESIUM $F_2$ AND PARENTAL LINES

| Parent or cross | Days from emergence to heading in one-day classes | | | | | | | | | | | | | | | Total number | Mean | Standard deviation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | | | |
| Reward | 3 | 6 | 3 | – | – | – | – | – | – | – | – | – | – | – | – | 12 | 52.0±0.21 | 0.71±0.15 |
| Caesium | – | – | – | – | – | – | – | – | – | – | 9 | 3 | – | – | – | 12 | 61.3±0.14 | 0.44±0.09 |
| Reward × Caesium | – | 4 | 7 | 19 | 23 | 29 | 18 | 14 | 10 | 4 | 3 | 1 | – | – | – | 132 | 56.1±0.18 | 2.11±0.13 |
| Marquis | – | – | – | – | 1 | 4 | 13 | 12 | – | – | – | – | – | – | – | 30 | 57.2±0.13 | 0.79±0.10 |
| Caesium | – | – | – | – | – | – | – | 1 | 5 | 9 | 12 | 3 | – | – | – | 30 | 60.4±0.18 | 0.94±0.12 |
| Caesium × Marquis | – | 5 | 35 | 97 | 186 | 280 | 278 | 224 | 150 | 76 | 37 | 24 | 16 | 3 | 1 | 1412 | 57.0±0.06 | 2.11±0.04 |

The $F_3$ distribution of Caesium × Marquis also approaches that of a normal curve, the mean being approximately equal to that of Marquis. Transgressive segregation for time of heading occurred beyond the extremes of both parents, but was, however, considerably more pronounced in the direction of Marquis. These data show a partial dominance of earliness and that genes for this character were contributed by both parental varieties. Polymeric factors are indicated as governing the inheritance of earliness in both crosses.

Five $F_3$ plants from each of 34 $F_4$ lines of the cross Reward × Caesium, were studied in the $F_4$ for earliness. The $F_3$ plants selected, from each $F_3$ line used, were representative of the earliest, intermediate and latest individuals in the line. The $F_3$ lines used were chosen at random. A correlation coefficient of $+.652$ was found between the individual $F_3$ plants and the corresponding $F_4$ lines, while an $r$ of $+.781$ was obtained for the correlation between the average of the five $F_3$ plants grown in the $F_4$ and the mean of the $F_3$ lines from which the plants were selected. The P values of both correlations exceeded the 1% point.

*Grain Yield*

Aamodt, Torrie and Wilson (6) have recently reviewed the literature on the inheritance of grain yield in wheat. The results of previous investigators show that grain yield is a definite heritable character, but that its expression is influenced to a large extent by environmental conditions.

The plant yield of grain in grams was studied in the $F_3$ and $F_4$ in the cross Reward × Caesium. The plants of each line were harvested separately. The heads were wrapped in cheesecloth to prevent loss in handling. The border plants, at both ends of each row, were noted but were not used in the yield studies. Each plant was threshed separately and the yield recorded in grams. The average grain yield per plant and standard deviation were calculated for each line.

The distributions of the mean grain yield per plant for the $F_3$ and parental lines of Reward × Caesium are shown in Table VIII. The standard deviation and standard errors were not calculated for the parental varieties, on account of the small number of lines used. The $F_3$ distribution is in the form of a

TABLE VIII

FREQUENCY DISTRIBUTION, MEAN AND STANDARD DEVIATION FOR GRAIN YIELD PER PLANT OF REWARD × CAESIUM $F_3$ AND PARENTAL LINES

| Parent or cross | Grain yield per plant in 0.5 gram classes | | | | | | | | | | | Total number | Mean | Standard deviation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5.8 | 6.3 | 6.8 | 7.3 | 7.8 | 8.3 | 8.8 | 9.3 | 9.8 | 10.3 | 10.8 | | | |
| Reward | – | 2 | 2 | 1 | – | – | – | – | – | – | – | 5 | 6.7 | — |
| Caesium | – | – | – | – | – | – | – | – | 1 | 1 | 3 | 5 | 10.5 | – |
| Reward × Caesium | 1 | 1 | 6 | 13 | 11 | 11 | 6 | 5 | 2 | 1 | – | 57 | 8.0 ± 0.12 | 0.93 ± 0.09 |

normal curve. The mean is inclined towards that of Reward, the low-yielding parent. The data suggest that polymeric factors are concerned in the difference between the yielding ability of Reward and Caesium.

Five $F_3$ plants from each of 15 $F_3$ lines, selected at random, were studied in the $F_4$. The $F_3$ plants, selected from each $F_3$ line, were representative of the lowest, intermediate and highest yielding individuals in the line. A value of $+.279$ was secured for the correlation coefficient between the grain yield of the individual $F_3$ plant and that of the corresponding $F_4$ line. A much more significant value of $+.648$ was obtained between the average of the plants grown in the $F_4$ and the mean of the $F_3$ line from which they were selected. The P value of the first correlation exceeded the 5% point, while that of the second exceeded the 1% point. The higher value of the second correlation can be explained largely by the fact that the average grain yield of all the plants within a line gives a much better indication of yielding ability than that of any individual plant. This is largely on account of the great variability in grain yield that occurs among the individual plants in a line.

## Correlated Inheritance

The relationships of the qualitative characters with each other, and with those of the quantitative characters, were measured by the $\chi^2$ test of independence or association. The association between any two characters was obtained by calculating $\chi^2$ and determining its value from Fisher's tables (14). Simple and partial correlation coefficients were used to study the relationships among the quantitative characters. Throughout these analyses a P value of .05 was taken as the level of significance.

Table IX gives the $\chi^2$ tests of independence or association among the qualitative characters studied in the $F_3$ of the crosses Reward × Caesium and Caesium × Marquis. The data in this table show that the characters are inherited independently.

TABLE IX

$\chi^2$ TESTS OF INDEPENDENCE OR ASSOCIATION AMONG THE QUALITATIVE CHARACTERS STUDIED IN THE $F_3$ OF THE CROSSES REWARD × CAESIUM AND CAESIUM × MARQUIS

| Characters compared* | Reward × Caesium | | Caesium × Marquis | |
|---|---|---|---|---|
| | $\chi^2$ | P lies between | $\chi^2$ | P lies between |
| Awning and glume color | 10.46 | .20 and .10 | 7.91 | .20 and .10 |
| Awning and glume pubescence | 7.28 | .20 and .10 | — | — |
| Awning and straw color | 5.53 | .30 and .20 | 4.94 | .30 and .20 |
| Awning and spike regularity | — | — | 2.73 | .70 and .50 |
| Glume pubescence and straw color | 4.63 | .50 and .30 | — | — |
| Glume pubescence and glume color | 3.63 | .80 and .70 | — | — |
| Straw color and glume color | 4.70 | .50 and .30 | 5.64 | .50 and .30 |
| Straw color and spike regularity | — | — | 0.99 | .95 and .90 |
| Glume color and spike regularity | — | — | 8.33 | .30 and .20 |

**The degrees of freedom used for the different comparisons were not necessarily the same.*

Aamodt and Torrie (3) have recently reviewed the literature on the relation between awning and grain yield in wheat. Certain investigators found an association between these two characters, while others found no association, depending both upon the material used and the environmental conditions under which it was grown. In the crosses Bobs × Propo, and Hard Federation × Propo, Clark, Florell and Hooker (11) found a positive relation between lodging and awn length. Goulden and Neatby (19) obtained no relation between awnedness and either straw strength or plant height, from a study of H-44-24 × Marquis lines in rod row trials. However, they secured a P value of .029 between awning and earliness. In a cross between the varieties Forward and Albit, Shen (26) found no indication of a linkage between awning with either plant height or date of heading.

Table X gives the $\chi^2$ tests of independence or association for the qualitative characters compared with the quantitative characters in the crosses Reward × Caesium and Caesium × Marquis. Straw color and earliness of heading showed definite indications of linkage in both crosses. In the $F_3$ and $F_4$ of the Reward × Caesium cross, the average number of days from seedling emergence to heading of the white strawed lines was respectively $1.6 \pm 0.53$ and $2.0 \pm 0.50$ days less than that for the purple strawed lines. The white strawed lines of Caesium × Marquis were $2.4 \pm 0.29$ days earlier than the purple strawed lines. In maturity the white strawed lines were $1.6 \pm 0.35$ days earlier than the purple strawed lines in the $F_4$ of Reward × Caesium. The average length of the white strawed lines was $3.6 \pm 0.80$ cm. shorter than the purple strawed lines in the $F_3$ of the cross Caesium × Marquis. No such relationship was found in the cross Reward × Caesium. This indicates that the genetical constitution of Reward and Marquis for plant height differs.

The awned $F_4$ lines of Reward × Caesium showed $4.3 \pm 1.7\%$ more lodging and were $1.0 \pm 0.44$ days earlier in heading than the awnletted lines, the P values in both of these cases being between .05 and .02. In the $F_3$ no significant association was found between these characters. For these reasons, the association found in the $F_4$ is not considered to be significant. The $F_3$ awned lines of Caesium × Marquis were $2.0 \pm 0.80$ cm. shorter than the awnletted lines. This difference is believed to be due to chance, for Caesium, the awned parent, is taller than Marquis, the awnletted parent. Additional factors for tallness could not have been contributed by Marquis as no transgressive segregation for plant height occurred in the $F_3$. No significant associations were found among the other characters studied.

Only a brief review of the results of other workers, concerning the relationships among the characters given in Table XI, will be made. Clark (10) Goulden and Elders (18), and Bridgford and Hayes (8) report significant positive correlations between grain yield and earliness. Significant negative correlations were obtained by Smith and Clark (27), Goulden and Neatby (19) and Waldron (31). No significant associations were found between these two characters by Clark and Hooker (12) and Hayes, Aamodt and Stevenson

TABLE X

$\chi^2$ TESTS OF INDEPENDENCE OR ASSOCIATION FOR THE COMPARISON OF THE QUALITATIVE WITH THE QUANTITATIVE CHARACTERS OF THE $F_3$ AND $F_4$ OF REWARD × CAESIUM AND THE $F_3$ OF CAESIUM × MARQUIS

| Qualitative characters | Quantitative characters | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lodging index | | Plant height | | Grain yield | | Days from emergence | | | |
| | | | | | | | To heading | | To maturity | |
| | $\chi^2$ | P lies between | $\chi^2$ | P lies between | $\chi^2$ | P lies between | $\chi^2$ | P lies between | $\chi^2$ | P lies between |
| | | | | | *$F_3$ Reward × Caesium* | | | | | |
| Glume color | 14.63 | .30–.20 | 12.73 | .20–.10 | 3.69 | .95–.90 | 13.93 | .50–.30 | — | — |
| Glume pubescence | 1.08 | .98–.95 | 8.06 | .20–.10 | 6.23 | .30–.20 | 8.57 | .50–.30 | — | — |
| Straw color | 5.40 | .80–.70 | 1.57 | .80–.70 | 5.68 | .30–.20 | 17.27 | .01–.00 | — | — |
| Awning | 8.70 | .50–.30 | 10.40 | .10–.05 | 7.65 | .30–.20 | 6.52 | .70–.50 | — | — |
| | | | | | *$F_4$ Reward × Caesium* | | | | | |
| Glume color | 2.50 | .50–.30 | 5.40 | .20–.10 | 2.60 | .30–.20 | 0.50 | .95–.90 | 0.73 | .90–.80 |
| Glume pubescence | 2.42 | .70–.50 | 2.53 | .50–.30 | 2.27 | .50–.30 | 4.94 | .10–.05 | 1.69 | .70–.50 |
| Straw color | 6.85 | .20–.10 | 7.11 | .10–.05 | 1.81 | .50–.30 | 21.41 | .01–.00 | 16.11 | .01–.00 |
| Awning | 9.09 | .05–.02 | 3.80 | .30–.20 | 1.61 | .50–.30 | 8.85 | .05–.02 | 6.65 | .10–.05 |
| | | | | | *$F_3$ Caesium × Marquis* | | | | | |
| Glume color | 15.68 | .20–.10 | 4.46 | .90–.80 | — | — | 7.18 | .70–.50 | — | — |
| Spike regularity | 12.70 | .20–.10 | 9.98 | .20–.10 | — | — | 11.81 | .20–.10 | — | — |
| Straw color | 5.01 | .80–.70 | 23.65 | .01–.00 | — | — | 63.78 | .01–.00 | — | — |
| Awning | 6.68 | .70–.50 | 14.43 | .05–.02 | — | — | 7.62 | .30–.20 | — | — |

*The degrees of freedom used for the different comparisons were not necessarily the same.*

(21). Between the characters of grain yield and plant height Clark (10), Clark and Hooker (12), Hayes, Aamodt and Stevenson (21), Goulden and Neatby (19) and Bridgford and Hayes (8) obtained significant positive correlations, while Waldron (31) found no association. Goulden and Elders (18) found weak straw to be associated with high grain yield. Correlation studies between earliness and plant height have shown different results. Clark (10) Hayes, Aamodt and Stevenson (21) and Goulden and Neatby (19), obtained significant positive correlations; Bridgford and Hayes (8) significant negative correlations; while Clark (10) and Clark and Hooker (12) report the lack of significant correlations between these two characters. Goulden and Elders (18) and Goulden and Neatby (19) found no significant association between earliness and lodging. A significant negative correlation was obtained between plant height and straw strength by Goulden and Neatby (19). In many of the cases reported above, the apparent contradictory results found can be explained by the fact that the investigators worked with different material grown under widely differing conditions.

TABLE XI

SIMPLE AND PARTIAL CORRELATION COEFFICIENTS AMONG THE QUANTITATIVE CHARACTERS OF THE $F_3$ AND $F_4$ OF REWARD × CAESIUM AND OF THE $F_3$ OF CAESIUM × MARQUIS

| Simple | | | Partial | | |
|---|---|---|---|---|---|
| Variables correlated† | Number | $r$ | Variables correlated | Number | $r$ |
| | | *$F_3$ Reward × Caesium* | | | |
| YH | 57 | +.101 | | | |
| YHe | 57 | +.162 | | | |
| YL | 57 | +.054 | | | |
| HHe | 132 | +.284** | HHe : L | 132 | +.290** |
| HL | 132 | +.191* | HL : He | 132 | +.161* |
| HeL | 132 | +.252** | HeL : H | 132 | +.253** |
| | | *$F_4$ Reward × Caesium* | | | |
| YH | 78 | −.239* | | | |
| YHe | 78 | +.121 | | | |
| YL | 78 | +.190 | | | |
| YM | 78 | +.042 | | | |
| HHe | 145 | +.560** | HHe : LM | 145 | +.124 |
| HL | 145 | +.142 | HL : HeM | 145 | −.219* |
| HM | 145 | +.782** | HM : HeL | 145 | +.677** |
| HeL | 145 | +.402** | HeL : HM | 145 | +.277** |
| HeM | 145 | +.676** | HeM : HL | 145 | +.376** |
| LM | 145 | +.327** | LM : HHe | 145 | +.207* |
| | | *$F_3$ Caesium × Marquis* | | | |
| HHe | 297 | +.379** | HHe : L | 297 | +.416** |
| HL | 297 | +.216** | HL : He | 297 | +.171** |
| HeL | 297 | +.291** | HeL : H | 297 | +.314** |

†*Y = grain yield in grams;* H = *days from emergence to heading;* He = *plant height;* L = *lodging index;* M = *days from emergence to maturity.*

*P *value exceeds 5% point.*

**P *value exceeds 1% point.*

Table XI gives the simple and partial correlations, among the quantitative characters studied, in the $F_3$ and $F_4$ of Reward × Caesium and in the $F_3$ of Caesium × Marquis. In the $F_3$ and $F_4$ of the cross Reward × Caesium, grain yield was not significantly correlated with any of the other characters, save with the one exception of earliness of heading in the $F_4$. In this case a significant negative correlation of $-.239$ was obtained. For these reasons the character grain yield was not included in the calculation of the partial correlations. Significant positive correlations were obtained in almost every other case. Between earliness of heading and lodging index significant positive correlations were obtained in the $F_3$ of both crosses. A significant negative correlation was found between these two characters in the $F_4$ of the cross Reward × Caesium. The selection of the $F_3$ plants, for $F_4$ planting, was at random. The differences in relationship found must be due either to a different effect of the environmental conditions of the two seasons, or to the holding of maturity constant in the calculation of the partial correlation in the $F_4$. The correlations between heading and height were significant in the $F_3$ of both crosses. In the $F_4$ of Reward × Caesium the second order partial correlation between these characters was positive but not significant. These correlations, although for the most part significant, show that the characters, with the exception of heading and maturity, are not closely dependent upon one another. The characters heading and maturity, which were studied only in the $F_4$ of Reward × Caesium, are shown to be closely related to one another.

## Ackowledgments

The author wishes to make grateful acknowledgment to Dr. O. S. Aamodt, former Professor of Genetics and Plant Breeding, University of Alberta, now Chairman of the Department of Agronomy, University of Wisconsin, for valuable assistance during the course of the investigation, and for reading and editing the manuscript; and to the National Research Council of Canada for financial assistance received.

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# Canadian Journal of Research

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VOL. 14, SEC. D. OCTOBER, 1936 NUMBER 10

## THE BLACKFLIES OF EASTERN CANADA (SIMULIIDAE, DIPTERA).[1] PART II.

BY C. R. TWINN

### Subgenus *Simulium* Latreille

*Simulium* Latreille, Hist. Nat. Ins. et. Crust., 3 : 426, 1802.
*Atratocera* Meigen, Klass., 1 : 94, 1804.
*Boophthora* Enderlein, D. Tier. Woch., 29 : 199, 1921.
*Chirostilbia* Enderlein, ibid.
*Edwardsellum* Enderlein, ibid.
*Gynonychodon* Enderlein, ibid.
*Odagmia* Enderlein, ibid.
*Wilhelmia* Enderlein, ibid.
*Gomphostilbia* Enderlein, ibid.
*Stilboplax* Enderlein, ibid.
*Friesia* Enderlein, Konowia, 1 : 69, 1922.
*Discosphyria* Enderlein, ibid., 1 : 72.
*Byssoden* Enderlein, Zool. Anz., 62 : 209, 1925.
*Pseudosimulium* Baranoff, Neue Beitr. Sys. Ins., 3 : 164, 1926.
*Pseudodagmia* Baranoff, ibid.
*Pternaspatha* Enderlein, Arch. Klass. phyl. Ent. 1 : 88, 1930.
*Anasolen* Enderlein, ibid., 1 : 94.
*Notolepria* Enderlein, ibid., 1 : 95. ;
GENOTYPE.—(*Culex colombaschensis* FABRICIUS) = *Culex reptans* LINNAEUS.

#### KEY TO THE FEMALES OF *Simulium*

(Based on external characters and genitalia)

1. Fore coxae black, grayish pollinose; mesonotum distinctly striped; abdomen opaque-gray with black markings . . . . . 2
   Fore coxae yellow; mesonotum not distinctly striped; abdomen dark-brown or black 3
2. With three mesonotal vittae; hairs on stem vein black; sub-costa with a ventral row of hairs . . . . . *Simulium pictipes* Hgn.
   With five mesonotal vittae; hairs on stem vein pale; sub costa bare . . . . . *Simulium vittatum* Zett.
3. Pleural tuft pale . . . . . 4
   Pleural tuft black . . . . . 6
4. Claws with a small sub-basal tooth; medium sized species close to *venustum;* anal lobe rounded quadrate, as long as ninth tergite, moderately setose posteriorly . *corbis* n. sp.
   Claws simple . . . . . 5

[1] *Concluded from September issue.*

5. Tergites six to nine black, shining; anal lobe strap-shaped, shorter than cercus (horizontally), and less than twice as wide, evenly setose; hairs on stem vein all pale . . . . . *venustum* Say
   Tergites six to nine grayish black, sub-opaque; anal lobe pyriform, narrow behind cercus, broadly rounded below, twice as long as cercus and twice as wide, anterior margin bare of setae; hairs on stem vein variable, pale and dark intermixed . . . . . *ottawaense* n. sp.
6. Sub-costa with a row of hairs on ventral surface; anal lobe not produced anteriorly; about same horizontal length as cercus; medium sized species . . *perissum* D. & S.
   Sub-costa bare; median area of anal lobe produced anteriorly to about twice length of cercus; smaller species . . . . . 7
7. Forks of genital rod tapered beyond tooth; hairs on stem vein all black . *fibrinflatum* n. sp.
   Forks of genital rod roundly expanded beyond tooth; hairs on stem vein variable, yellow, bronzy-brown, black, or the pale and dark hairs mixed . *nigroparvum* n.sp.

## KEY TO THE MALES OF *Simulium*

### (Based on the genitalia)

1. Clasper with three terminal teeth or none . . . . . 2
   Clasper with a single terminal tooth, or spine . . . . . 3
2. Clasper shorter than side-piece, with three terminal teeth; adminiculum arched . . . . . *vittatum* Zett.
   Clasper longer than side-piece, terminal teeth absent; adminiculum deeply cleft mesially . . . . . *pictipes* Hgn.
3. Adminiculum narrow, inverted Y-shaped, with posterior process or keel . . . 4
   Adminiculum broader, tooth-shaped, without posterior process . . . . . 5
4. Posterior process apically rounded, marginally denticulate . . . . *corbis* n. sp.
   Posterior process rounded, smooth, setulose, without marginal denticles . *ottawaense* n. sp.
5. Clasper with a small inner basal process; prongs of adminiculum simple . . . 6
   Clasper simple; prongs of adminiculum each with a posterior sub-basal arm . . 7
6. Basal process of clasper smooth; side-piece with rather numerous setae; adminiculum quadrate . . . . . *venustum* Say
   Basal process spinose; side-piece setose only on distal margin; adminiculum sub-quadrate, apically emarginate and denticulate . . . . . *perissum* D. & S.
7. Adminiculum one and one-fourth times as long as broad, upper margin denticulate at sides, hirsute medially . . . . . *fibrinflatum* n. sp.
   Adminiculum twice as long as broad, upper margin denticulate throughout, hairs sparse or absent . . . . . *nigroparvum* n. sp.

### *Simulium (Simulium) vittatum* ZETTERSTEDT

*Simulium tribulatum* Lugger, 2nd. Rep. Ent. Minn. p. 205, 1896.

*Simulium glaucum* Coquillett, Proc. U.S. Nat. Mus., Vol. 25 : 97, 1903.

*Simulium dahlgruni* Enderlein, Deut. Tierarz. Woch. Hanover, 1921, p. 43; zool. Anz. Vol. 53, p. 45.

*Female.*—Length, 2–3 mm.; wing, 2.8–4 mm. Integument black, covered with opaque gray pollinosity, and brown to black markings; pubescence pale, rather sparse. *Head:* opaque-gray; front broad, with sparse pale hairs; clypeus as broad as long, about as wide as front at vertex, hairs longer, more numerous. Antenna 11-jointed, the two basal joints brownish, remainder black, tapering; palpi black. *Thorax:* mesonotum with five dark-brown to black vittae; pubescence short, sparse, pale; scutellum with a few recumbent pale scales and numerous upright pale hairs; postnotum silvery pollinose with

a central dark spot; pleural tuft pale. *Wings:* hairs on stem vein and base of costa pale, remainder black; those on costa and a row on the outer part of radial vein mixed with spinules; sub-costa bare; radius bare between stem vein and base of radial sector; sector simple, reaching the costa close to the tip of radial vein, lower surface with a row of hairs; basal cell absent; halteres pale, yellowish, the base of stem black. *Legs:* black, except the basal half of tibiae and mid and hind basitarsi which are yellowish; fore coxae gray pollinose; fore tibiae with a basal pollinose white patch; fore tarsi slightly flattened; hind basitarsus about 6 × 1, and two-thirds the width of tibia; calcipala small; pedisulcus distinct; claws simple. *Abdomen:* opaque-gray, with three rather indefinite rows of opaque black spots on the dorsum, the median row often coalescing to form a central black stripe; basal fringe and general, short, sparse pubescence, pale; chitinization of tergites three to five reduced to small median plates; sternites two to seven entirely membranous. *Genitalia* (Fig. 10, 4, 5): valves of ovipositor membranous. Anal lobe rather sparsely setose, about as long as cercus, but twice as wide (ventral measurement), one-half its width conically lobed ventrad of cercus and concealing the ovipositor; lower margin clear and setulose. Cercus rounded distally; setose. Arms of genital rod quadrately expanded, the corners produced; chitinization of outer portion denser than inner.

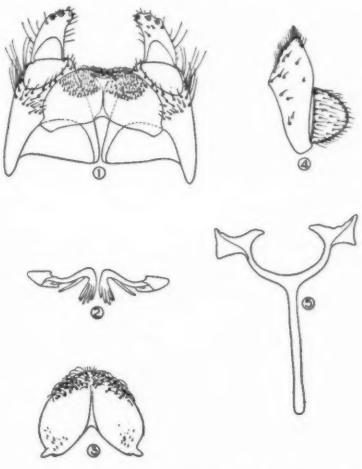

FIG. 10. *Parts of genitalia of Simulium (Simulium) vittatum Zett.: male (1) side-pieces, claspers and adminiculum, (2) adminiculum arms, (3) adminiculum detached; female (4) anal lobe and cercus (ventral side up), (5) genital rod.*

*Male.*—Distinctly darker than female. Antenna entirely black, more slender. Mesonotum white or silvery pollinose on lateral and posterior margins; disk velvety black and bearing two short, rather indefinite pale stripes; pilosity hair-like, moderate, yellowish. Hairs on stem vein and base of costa black. Legs as in female, but darker, the hairs longer, especially on the fore femora and posterior margins of the hind legs. Abdomen opaque black with silvery pollinose patches on the sides; basal fringe dark-brown to black, other pilosity pale, rather long; none of the tergal plates greatly reduced; only the second sternite entirely membranous. *Genitalia* (Fig. 10, 1-3): side-piece longer than wide, conical; clasper shorter than side-piece, obliquely truncate, with three terminal teeth. Adminiculum broad, convex above, emarginate below; rather weakly chitinized, hirsute, basal prongs abbreviated. Adminiculum arms chitinous, laterally expanded; inner margin with elongate teeth.

*Pupa* (Plate I, Fig. 6).—Length 3–5 mm.; the respiratory filaments about three-fourths that of the pupa. The respiratory tuft consists of a short main trunk which gives rise to eight branches, each again dividing to produce sixteen filaments in all. The cocoon is of the usual wall-pocket type.

*Notes.*—This blackfly is one of the most common species in the Ottawa district, both in Ontario and Quebec, and occurs in many of the smaller rivers and streams in that area, often in great abundance, and usually associated with *Simulium venustum*. It attacks domestic animals, particularly horses, and occasionally man. There are two or three generations a year, the broods overlapping, so that during the summer months all stages may commonly be found together. I have collected pupae as early as April 12, and as late as the end of September. The winter is spent in the larval stage. Larvae taken from a small stream by breaking through the covering of ice and snow, on Dec. 15, 1931, were mostly well grown, although some were barely one-third grown. In all of them the histoblasts were undeveloped. Despite the low temperature they were feeding, as was evidenced by an examination of the gut contents.

The eggs and mature larvae of *vittatum* are shown on Plate I, Figs. 1 and 3. The pupae and cocoons are depicted on Plate I, Fig. 6 (opposite page 100).

*Distribution.—S. vittatum* is extremely widely distributed over North America, from Mexico to the Arctic, and from the Atlantic to the Pacific. It is a common species in Europe where it was originally described, probably from Lapland; it also occurs in Greenland. I have found the immature stages in most of the rivers and streams in the Ottawa district, wherever blackflies develop, and have reared adults of both sexes from pupae taken from streams in the wooded Gatineau hills, in Quebec, and in the more level country in Ontario, south of the Ottawa river. In addition, there are specimens in the National Collection from the following localities in Ontario: Nipigon, 1. VII. 23 (M. K. Bigelow); Biscotasing, 9. VII. 30 (K. E. Schedl); Dundonald ("in horse's ear") 27. VIII. 00; Trenton, 20. VI. 01 (Evans); Strathroy (attacking horses and cows), 22. VIII. 32, (H. F. Hudson); Toronto, 27. VII. 28 (C. R. Twinn); Algonquin Park, 18. V. 32 (C. R. Twinn). W. J. Brown collected seven females at Lake Harbour, Baffin island, August 9–14, 1935.

### *Simulium (Simulium) pictipes* HAGEN

*Simulium pictipes* Hagen, Proc. Bos. Soc. Nat. Hist., 20.: 305, 1879.

*Simulium innoxium* Comstock, Manual for the Study of Insects, p. 452, 1895.

*Female.*—Closely allied to *vittatum* Zetterstedt, from which it may be separated by the following characters: Length, 3–4 mm.; thorax with three mesonotal vittae; upright hairs on the scutellum and the hairs on the stem vein of the wing black; lower surface of the sub-costa with a row of fine hairs; abdomen darker, the opaque black markings on the dorsum undivided. *Genitalia* (Fig. 11, A, 4, 5): ovipositor valves narrow, thin, membranous. Anal lobe scarcely larger than cercus, rounded quadrate, broadly conical

ventrally; chitinized, especially along anterior margin, a group of spicules anterior to this; ventral and posterior margins setulose. Cercus rounded quadrangular, wider than long, setose. Arms of genital rod triangularly divaricate, the apical expanded portions chitinized and singly toothed.

*Male.*—Similar to male of *vittatum* Zett., but larger; two basal joints of antenna brownish; palpi about one-third longer than antenna; legs generally darker, base of hind tibiae not broadly pale; abdominal basal fringe paler, yellowish-brown; sub-costa bare. *Genitalia* (Fig. 11, A, 1-3): side-piece

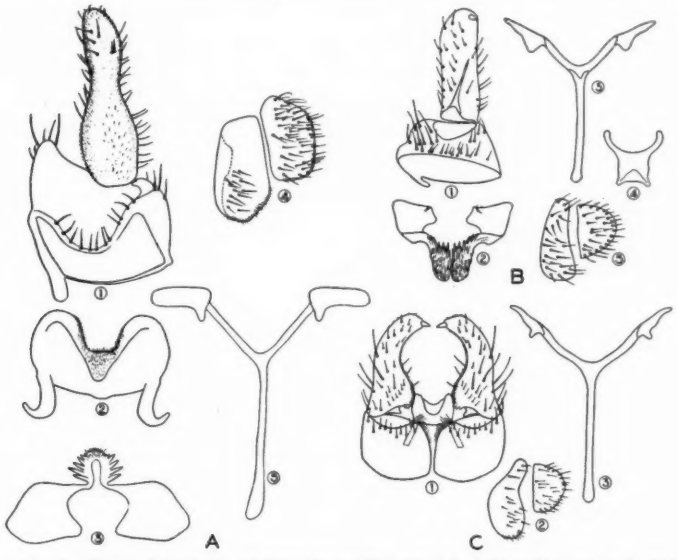


FIG. 11. *A. Parts of genitalia of Simulium (Simulium) pictipes Hagen: male (1) side-piece and clasper, (2) adminiculum, (3) adminiculum arms; female (4) anal lobe and cercus, (5) genital rod. B. Parts of genitalia of Simulium (Simulium) venustum Say: male (1) side-piece and clasper, (4) adminiculum, (2) adminiculum arms (4 and 2 ventral side up); female (3) genital rod, (5) anal lobe and cercus. C. Parts of genitalia of Simulium (Simulium) perissum D. & S.: male (1) side-pieces, claspers and adminiculum; female (2) anal lobe and cercus, (3) genital rod.*

quadrate, outer apical angle produced; clasper longer than side-piece, rounded and somewhat constricted at middle, without terminal tooth. Adminiculum weakly chitinized, nearly twice as wide as long, broadly and deeply cleft mesially, minutely hirsute; basal prongs more strongly chitinized, tapering. Adminiculum arms with a few long, pointed teeth; lateral plates broad.

*Pupa.*—Length 3.5–5 mm. The respiratory tuft is about two-fifths as long as the pupa, and consists of nine rather stout filaments, eight of them arranged in pairs on very short stalks and the ninth arising directly from the short main trunk. The cocoons are boot-shaped, with open weave, and the anterior margin not thickened. The pupa and its respiratory filaments lie concealed within the cocoon.

*Notes.*—I have found the immature stages of this species only in very swift water such as in, or just below, waterfalls. Masses of the pupae and large numbers of the distinctive black larvae, in various stages, were discovered under the cascading water on the steep rock face of a waterfall of the Blanche river (Fig. 7, B), at Perkins Mills, Que., on August 24, 1932. Egg masses were also plentiful in the shallow water along the sides of the falls in places where the rock was continually wetted by the spray, or by the intermittent rise and fall of the water level. Adults were flying above the falls; specimens captured were all males. Again, on July 26, 1935 (9–10 a.m.), large numbers of males were seen and many netted at the foot of another waterfall, several miles south of Perkins Mills (Fig. 12, A). These were in erratic flight from just above the surface of the swift and broken water to a height of probably 15–20 feet.

Adults of both sexes were reared on July 26, 1935, from pupae taken with difficulty from the smooth rock surface beneath the tumultuous waters of the falls of the Blanche river, at Perkins Mills; also from pupae found on August 2, 1935, in crevices in the masonry near the foot of a concrete dam in the Mississippi river, at Carleton Place, Ont. (Fig. 9, B).

Smart (48, p. 62) who studied the biology of this species at Ithaca, N.Y., found that it passes through four (possibly five) generations in a year, the winter being passed in the larval stage. The number of generations annually is probably fewer in our more northern latitude. Malloch (29, p. 57) states that the female will bite horses, and Jobbins-Pomeroy (38, p. 6) records the removal of an engorged female from a mule's ear. Smart induced specimens in captivity to bite his arm. Apparently, however, it does not normally attack humans.

*Distribution.*—It has been recorded hitherto only from the Eastern United States. Malloch (29, p. 56) and Dyar and Shannon (5, p. 28) list records from New York, Maryland, District of Columbia, Virginia, South Carolina, Indiana and Minnesota. My records are all from the Ottawa district including rearings and collections at Carleton Place, Ont., and Perkins Mills, Que. There are two specimens in the National Collection labelled Ottawa, 30. VII. 12 (Beaulieu) and 7. VIII. 23 (C. H. Curran). Its distribution in Eastern Canada is probably much wider than is indicated by these records.

### *Simulium (Simulium) venustum* Say

*Simulium venustum* Say, Journ. Acad. Sci. Phila. 3 : 28, 1823.
*Simulium molestum* Harris, Ins. Injur. to Veg., ed. 3 : 601, 1862.
*Simulium piscicidium* Riley, Amer. Ent. 2 : 367, 1870.
*Simulium irritatum* Lugger, 2nd. Rep. Ent. Minn. p. 177, 1896.
*Simulium minutum* Lugger, ibid.
*Simulium jenningsi* Malloch (part), U.S. Dept. Agr. Bur. Ent. Tech. Ser. No. 26, p. 41, 1914.
*Simulium austeni* Edwards, Bull. Ent. Res. 6 : 33, 1915.
*Simulium rileyana* Enderlein, Konowia, 1 : 75, 1922.

*Female.*—Length, 2–2.5 mm.; wing, 2.5–3 mm. *Head:* black; front glossy black with slight white pollinosity, as broad at vertex as clypeus; clypeus opaque white pollinose, as broad as long; antenna 11-jointed, black, basal joints reddish-brown; palpi about as long as antennae. *Thorax:* shining black, the mesonotum with whitish to brownish pollinosity, strongest at the margins, also on the pleurae; pubescence short, fine, yellowish; scutellum dark-brown, with a few recumbent pale scales and numerous upright brown to black hairs; postnotum bare, shining pollinose, a narrow silvery band along the anterior margin; pleural tuft pale. *Wings:* hairs on the stem vein and base of costa pale; remainder pale to dark, those on costa and a row on the outer part of radial vein mixed with spinules; sub-costa with a row of hairs on lower surface, terminating before apex; radius bare between stem vein and base of radial sector; the sector simple, reaching the costa close to tip of radial vein, lower surface with a row of hairs; basal cell absent; halteres pale yellowish, the base of stem black. *Legs:* largely yellowish with darker areas, notably the mid and hind coxae, the tibiae apically, the fore tarsi and terminal joints of mid and hind tarsi; all tibiae silvered on dorsal surfaces; pilosity pale to dark, moderate; fore tarsi flattened; hind basitarsus approximately 6 × 1 and two-thirds width of tibia; calcipala and pedisulcus present; claws simple. *Abdomen:* basal fringe pale yellow, general pubescence short, sparse, brown; segments three to five opaque velvet black; second segment silvery at the sides; remaining segments dark-brown to black, shining; tergal plates of segments three to five greatly reduced in width; sternites of all but the terminal segments membranous. *Genitalia* (Fig. 11, B, 3, 5): ovipositor valves small, broader than long, truncate, minutely setulose, and with a group of setae; membranous, weakly chitinized medially. Anal lobe dorsally one-half as long as cercus, increasing in length ventrally and truncately produced; setose throughout. Cercus rounded distally; evenly setose. Arms of genital rod divaricate, the more thickly chitinized expanded portions each with a broad, blunt tooth, and irregular ventral margin.

*Male.*—Mesonotum opaque black, with a posterior and lateral marginal band of light-reflecting silver-gray pollinosity, most conspicuous anteriorly where it forms two backward and inwardly directed silvery stripes which stop short of the middle of the disk; pubescence yellowish-brown; pleural tuft black. Hairs on stem vein and other hairs black; sub-costa bare. Legs black; basal half of anterior tibiae yellowish, dorsal surface with a white pollinose patch; mid and hind tibiae yellow narrowly at base, the dorsal surfaces faintly white pollinose and pale haired; fore coxae, base of mid basitarsus and bases of hind basitarsus and second joint, yellow; fore coxae with white dusting; hind tibia and basitarsus slightly inflated; basitarsus about 5 × 1 and two-thirds width of tibia. Abdomen opaque black, with more or less conspicuous ventro-lateral pearlaceous pollinose markings on segments two, six and seven; basal fringe and other pubescence dark-brown to black; tergal plates not greatly reduced; second sternite membranous, others with small chitinized plates. *Genitalia* (Fig. 11, B, 1, 2, 4): side-piece

rounded quadrate, wider than long, setae rather numerous. Clasper about twice as long as side-piece; stout, rounded, slightly constricted beyond the middle, a small basal projection on inner side, a spine at tip. Adminiculum stout, quadrate, strongly chitinized; basal prongs divaricate, incurved near tips. Adminiculum arms with numerous long teeth mixed with fimbriae; widening, angled, and broad at base.

*Pupa.*—Variable in size; respiratory tuft about one-half as long as the pupa, and consisting of six filaments arranged in three pairs on short stalks held in the vertical plane, the stalks sub-equal and somewhat variable in length. The cocoon is of the usual wall-pocket type, closely woven, with a thickened anterior margin (Plate I, Fig. 5). The pupa and other immature stages have been described and figured by several authors including Malloch (29), Jobbins-Pomeroy (38), Edwards (7), Puri (41), and Johannsen (23, 24).

*Notes.*—*S. venustum* is probably the most common and abundant species in the Ottawa district, on both sides of the Ottawa river, in Ontario and Quebec. It is found in all types of bodies of running water, permanent and temporary; in rivers, streams, rills and drainage ditches. Pictures of such breeding places are shown in Figs. 2, 3, 4, and 7, A. The immature stages commonly occur in association with other species attached to rocks, stones, aquatic plants, logs, debris, and other obstructions in the water. The larvae are often found in remarkable numbers: 734 in various stages of development were counted on an area of approximately one square inch on a submerged tree branch in Cranberry creek, Algonquin Park, on May 18, 1932; another count on a similar area revealed 742. The adult flies are found on the wing from May to October, but occur in greatest numbers in June and early July. There are probably three or four generations of the flies in a year; the various generations overlap, and during the summer all stages may be found together. The winter is passed in the larval stage. The eggs are illustrated on Plate I, Fig. 2, and the cocoons and pupal skins on Plate I, Fig. 5. This species is a troublesome pest of animals and man, and serves as the intermediate host of a protozoon blood parasite of ducks, *Leucocytozoon anatis* Wickware, as recorded by O'Roke (34). O'Roke found that this parasite caused a mortality rate of 10–100% in ducklings, but for adult ducks less than 1%. The disease is common in Canada as I have recorded elsewhere (55).

*Distribution.*—*S. venustum* occurs in Europe and throughout North America. It has been taken in many localities in every province in Canada. A detailed account of its distribution in the Dominion was published in 1933 (55), and need not be repeated here.

### *Simulium (Simulium) perissum* Dyar and Shannon

*Simulium perissum* Dyar and Shannon, Proc. U.S. Nat. Mus., Vol. 69, Art. 10, p. 43, 1927.

*Simulium jenningsi* Malloch (part), U.S. Dept. Agr. Bur. Ent. Tech. Ser. No. 26, p. 42, 1914.

Dyar and Shannon confined their description of *perissum* almost entirely to the genitalia of both sexes. Among adults of *venustum* reared from pupae collected in the Jock river, near Ottawa, Ont., on May 12, 1935, I found a male and female with genitalia which appear identical with those described and figured for *perissum*. The only external characters mentioned by Dyar and Shannon are contained in the statement quoted as follows: "A medium sized species, very close to *venustum* and very difficult to separate from the typical form. It is somewhat darker, with evidence of three mesonotal vittae; the stem vein is black pilose." I can find no trace of mesonotal vittae in my specimens. They differ from *venustum* as follows.

*Female.*—Somewhat darker; the antennae black, with basal joints faintly reddish; front sharply narrowed from vertex to base of antennae, sides strongly divergent; pleural tuft black; hairs on stem vein and base of costa black. *Genitalia* (Fig. 11, C, 2, 3): ovipositor valves broader than long, remote at tips; membranous, weakly chitinized along inner margins; minutely setulose, with one or two fine setae basally. Anal lobe with rather broad median area; moderately and irregularly setose. Cercus distally rounded; evenly setose. Arms of genital rod divaricate; the thickly chitinized part of outward expansions with a long basal tooth and irregular margin.

*Male.*—Generally darker in appearance than *venustum;* pollinose areas less conspicuous; legs almost entirely black, the pale portions of the hind tarsal joints indistinct; hind legs much inflated, the hind basitarsus slightly more than 3 × 1, and three-fourths the width of tibia. *Genitalia* (Fig. 11, C, 1): side-piece rounded quadrate, broader than long; setose only on distal margin. Claspers twice as long as side-piece, with an inward, basal, spinose projection and a slender terminal tooth. Adminiculum strongly chitinized, sub-quadrate, wider than long, apically emarginate, denticulate; basal prongs stout, divaricate, truncated. Adminiculum arms with long close teeth mixed with fimbriae; moderately broad basally.

*Pupa.*—The species was reared from a collection of pupae with six respiratory filaments, thought to be *venustum*. Both species emerged from these pupae. It will probably be necessary to rear *perissum* from isolated pupae to establish the presence of specific differences in the latter, if such are present.

*Notes.*—Two females and six males emerged with numerous adults of *venustum* on May 14–16, 1935, from pupae collected on submerged stones, tufts of grass and twigs in the Jock river, Ont. (Fig. 12, B), at a point about ten miles from Ottawa, where it enters the Rideau river. The Jock river is shallow and has a rocky bed thickly strewn with rocks and boulders. During the summer it often largely dries up in places, but in early May it was flowing swiftly over the algae-covered rocks. The temperature of the water was 62° F. I failed to find specimens among the adults of *venustum* reared from eight other rivers and streams in the Ottawa district.

*Distribution.*—Jock river, near Ottawa, Ont. Dyar and Shannon (5, p. 44) recorded the species from Maryland, South Carolina and Virginia.

FIG. 12. *A. Rapids of Blanche river below falls, south of Perkins Mills, Que. Males of S. pictipes Hgn., we caught swarming here. B. Jock river, near Ottawa, Ont. S. venustum Say, S. perissum D. & S. and S. vittatum Zett., develop here.*

*Simulium (Simulium) fibrinflatum* N. SP.

*Female.*—Length about 2 mm.; wing, 2.5 mm.; closely allied to *venustum* Say, from which it differs as follows: palpi distinctly longer than antennae (possibly variable); pollinosity much less thickly dusted on front and mesonotum, the integument showing through glossy black, that on the pleurae more silvery; pleural tuft black; hairs on stem vein and base of costa black; sub-costa bare; the pollinose patches on the dorsal surfaces of all tibiae more conspicuous, silvery; terminal abdominal segments glossy black. *Genitalia* (Fig. 13, A, 3, 5): ovipositor valves short, wider than long, weakly chitinized, setose, blunt tips remote. Upper part of anal lobe short, expanding medially to twice the length of cercus; narrowing, bluntly rounded and slightly produced ventrally; rather sparsely setose along posterior margin and on ventral portion. Cercus rounded quadrate; setose. Genital rod similar to *venustum*, but the tooth on chitinized expanded portion of arms longer, more slender, bluntly pointed.

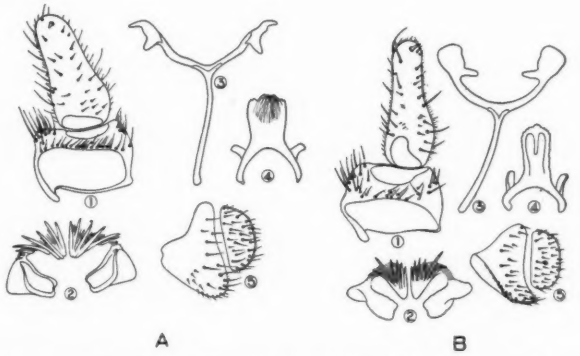


FIG. 13. *A. parts of genitalia of Simulium (Simulium) fibrinflatum n. sp.: male (1) side-piece and clasper, (4) adminiculum, (2) adminiculum arms; female (3) genital rod, (5) anal lobe and cercus. B. Parts of genitalia of Simulium (Simulium) nigroparvum n. sp.: male (1) side-piece and clasper, (4) adminiculum, (2) adminiculum arms; female (3) genital rod, (5) anal lobe and cercus.*

*Male.*—Similar to *venustum*, but with the following differences: pollinose band on margins of mesothorax and extending backward on disk more brilliant, iridescent anteriorly, and pearlaceous posteriorly; radial sector of wing bare of hairs except sparsely apically where it joins costa; legs paler, scarcely darker than in female: fore coxae, trochanters, femora except at apices, and the basal portions of first and second joints of mid and hind tarsi yellow, elsewhere black; the pollinose patches on dorsal surfaces of fore tibiae and bases of mid and hind tibiae conspicuous, silvery; joints of hind legs similar to female, none inflated. *Genitalia* (Fig. 13, A, 1, 2, 4): side-piece rounded quadrate, wider than long; clasper twice as long as side-piece, stout, somewhat swollen near base, a spine at tip. Adminiculum tooth-like, one-fourth longer than broad, strongly chitinized, especially the margins and basal prongs; hirsute medially; upper margin laterally denticulate; basal prongs stout, moderately divaricate,

each with a short, posterior curved, sub-basal arm. Adminiculum arms with an open row of long pointed teeth against a strigate membrane; more or less pocket-shaped laterally, strigate, the inner margin more thickly chitinized.
*Holotype.*—Male, Ottawa, Ont., 17. VIII. 35 (reared). Can. Nat. Coll.

*Allotype.*—Female, same data.

*Paratypes.*—Fourteen females, nineteen males, 23–25. VII. and 17–19. VIII. 35 (reared).

*Pupa.*—Length about 3 mm.; the respiratory tuft about one-fourth as long as the pupa; the filaments in the tuft six in number, short, stout, inflated, with the appearance of a cluster of minute, semi-transparent, sausage-shaped balloons, almost sessile, branching off in the vertical plane from a short base. Cocoon about 3.5 mm., somewhat slipper-shaped, rather closely woven, with a narrow, elongate-oval, window-like aperture in each side near the anterior border. The pupa lies completely within the cocoon, the respiratory tufts opposite the "windows".

*Notes.*—The characteristic pupae were found for the first time on July 22, 1935, in the Remic rapids (Fig. 14, A), of the Ottawa river, at Cunningham island. The exact location (not shown in illustration) was a narrow channel dividing the east end of the island from a tiny islet through which the shallow water was rushing at a speed of about five feet per second, over a shale-rock bottom. The temperature of the water was 76° F. The infestation was largely confined to the submerged branches and leaves of sweet gale, a shrub growing in profusion along the margins of the channel, and was mixed with two other species, *nigroparvum* and *venustum*, the former predominant. Large numbers of the three species had emerged, as was revealed by the empty pupal skins. Observations had been made at this point at intervals during the spring and summer, and on the most recent visit (July 3) no pupae of *fibrinflatum* were seen, although it is probable the species was present in the larval stage. On August 16, *fibrinflatum* predominated to a striking extent, only occasional pupae of *nigroparvum* and *venustum* being found. The speed of the water had dropped to about 3.5 feet per second, and the temperature was unchanged. On August 29, the infestation consisted chiefly of larvae of the three species; a rare pupa was found on Sept. 13 (water temperature 64° F.), and none at all on Sept. 30 (water temperature 56° F.), the date of the last visit, when only very young larvae were found. Numbers of adults of both sexes were reared from the pupae collected on July 22 and August 16.

### *Simulium (Simulium) nigroparvum* N. SP.

*Simulium venustum* var. *jenningsi* Twinn (not Malloch), Can. Ent. Vol. LXV, p. 3, 1933.

*Female.*—Average length 1.6 mm.; wing, 2.3 mm. A very small, glossy black species, similar to *venustum* in all other respects except as follows: general pollinosity less opaque; front glossy black; clypeus grayish pollinose, sub-opaque; mesonotum glossy black with faint grayish pollinosity, strongest

at the margins; pubescence yellowish-brown, rather sparse, scattered, hair-like; scutellum black, marginal hairs black, recumbent pale scales absent; pleural tuft black; hairs on stem vein variable, yellow, bronzy-brown or black, or the pale and dark hairs intermixed; sub-costa bare*; terminal abdominal segments darker and more glossy. *Genitalia* (Fig. 13, B, 3, 5): ovipositor valves small, remote, weakly chitinized; setose, especially on the inner margins, which are also somewhat embrowned. Anal lobe triangular, the postero-ventral angle slightly produced posteriorly behind cercus; chitinized, moderately setose, the anterior part of basal margin clear. Cercus rounded quadrate, wider than long; setose, arms of genital rod chitinized, roundly expanded, and with a long slender tooth.

*Male.*—Similar in size to female, but much darker. It differs from the male of *venustum* as follows: mesonotum opaque velvet-black, the marginal pollinose band which terminates on the disk, silvery or pearlaceous, somewhat iridescent anteriorly; pilosity dark-brown, almost black; radial sector bare, except close to the apical margin at the junction with the costa; legs predominantly black, the pollinose patches on tibiae silvery; hind basitarsus about 6 × 1, and three-fifths the width of tibia. *Genitalia* (Fig. 13, B, 1, 2, 4): similar to *fibrinflatum*. Side-piece rounded quadrate, wider than long; clasper twice as long as side-piece, a slender spine at tip. Adminiculum tooth-like, twice as long as broad, densely chitinized, centrally paler, strigate; upper margin denticulate; basal prongs stout, divaricate, truncate, each with a posterior sub-basal arm. Adminiculum arms with several long slender teeth mixed with fimbriae; lateral plates broad, darkly strigate.

*Holotype.*—Female, Ottawa, Ont., 10. VI. 35 (reared). Can. Nat. Coll.

*Allotype.*—Male, same data.

*Paratypes.*—Seven females, nine males, Ottawa (Remic rapids), 10–18. VI. 35; one female, five males, near Perkins Mills, Que. (Blanche river), 13. VI. 35, and one male, 26. VII. 35; two females, three males, Ottawa (Rideau river), 6–7. VI. 35. All reared from pupae.

*Pupa.*—Average length 2.5 mm. Respiratory tuft about one-fourth the length of the pupa, consisting of ten filaments. These are arranged as follows: two main trunks arise in approximately the same plane (vertical) from a short base; the lower trunk bears two branches, each of which gives rise to three filaments, a single filament basally and a pair distally; the upper branch divides twice near the base to form two pairs of filaments, thus making ten in all. The cocoon measures about 3 mm., and is similar to that of *fibrinflatum*, slipper-shaped, with a "window" anteriorly on each side.

*Synonymy.*—Johannsen (23, p. 381) described a species of blackfly under the name *S. venustum* var. a, which was reared from larvae and pupae with ten respiratory filaments taken from Fall creek, Ithaca, N.Y. The adults were 1.5 mm. long, with the terminal abdominal segments shining black.

**A specimen netted near Hull, Que., September 30, 1936, had four hairs in a row on the sub-costa.*

Johannsen's figure of the pupa shows the arrangement of the respiratory filaments different from that of my specimens. The submentum of the larva of *nigroparvum*, however, is similar to that of Johannsen's var. a, and has the ventral setae three in one marginal row and four in the other. Malloch (29, p. 42) in describing *jenningsi* mentioned that among the material were adults reared from pupae at Friersons Mill, La., which were in all probability the species described by Johannsen. This seems doubtful, as Jobbins-Pomeroy (38, p. 24) described and figured the pupa of *jenningsi* as having eight respiratory filaments: these pupae were determined from adults reared from them and compared with the type. Moreover, Dyar and Shannon (5, pp. 34, 43, 45), after examining Malloch's material in the United States National Museum, showed *jenningsi* to be a composite species made up of *venustum* Say, *slossonae* D. & S., and *perissum* D. & S. My material is specifically distinct from all of these. The species erroneously recorded by me (55, p. 3) as *venustum* var. *jenningsi* Malloch, from the Remic rapids of the Ottawa river, is *nigroparvum*.

*Notes.*—Pupae and larvae of *nigroparvum* were first found in the Remic rapids (Fig. 14, A), of the Ottawa river, close to the river bank on the Quebec side, near Hull, on June 25, 1931, attached to a round, smooth pole caught among the rocks in the rapids. They were next found with larvae in the same rapids on Sept. 16, 1932, on leaves and twigs of sweet gale growing on the eastern shore of Cunningham island, and hanging in the fast-running water. Numerous adults of both sexes emerged within a day or two from specimens placed in a sealer.

During 1935, visits were made to the Remic rapids twice or thrice a month from late April to the end of September. Recently transformed pupae were found on slabs of shale in the rapids on June 6 (water temperature 63° F.), and adults emerged from these June 8–10. By June 14, large numbers of empty pupal skins of this species were observed in the river; many pupae were collected and several score that emerged were preserved in alcohol. The pupae again began to appear early in July, and by July 22 (water temperature 76° F.) empty pupal skins predominated and may have marked the end of the second generation. A few pupae were found with those of *venustum* and *fibrinflatum* during visits on August 2 and 16. On August 29 (water temperature 70° F.), *S. nigroparvum* predominated in numbers over the other two species, but the pupae were not very abundant: on Sept. 13, only one pupa was found after a 40-minute search; on Sept. 30 (water temperature 56° F.) very young larvae, the species of which was not determined, were quite numerous, but no mature larvae or pupae could be found. It would seem from these observations that there are at most three generations of *nigroparvum* annually in the Ottawa region, the winter probably being spent in the immature larval stage.

Adult flies were also secured from pupae collected in the Hog's Back rapids of the Rideau river near Ottawa, Ont., June 5, 1935, and from submerged stones and grasses in the rapids of the Blanche river (Fig. 7, A), south of Perkins Mills, Que., on June 12, July 26 and August 18, 1935.

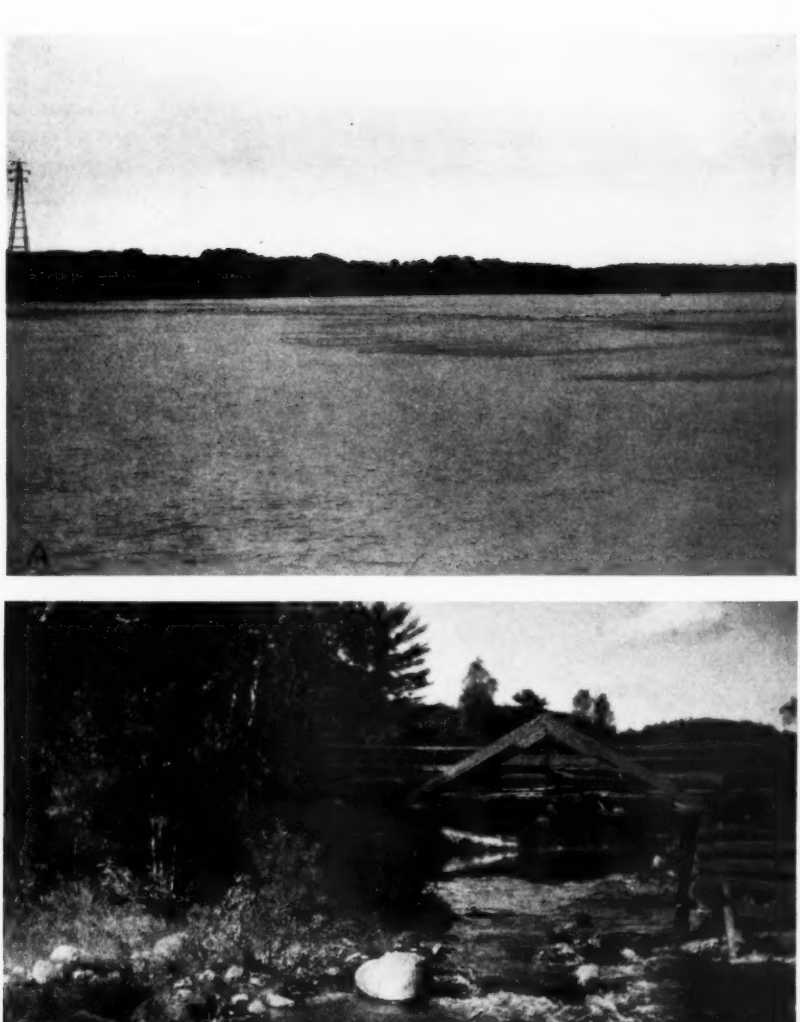


FIG. 14. *A. Remic rapids, Ottawa river; breeding place of S. venustum Say, S. nigroparvum n. sp., S. fibrinflatum n. sp. and S. vittatum Zett. B. Blanche river, north of Perkins Mills, Que.; S. venustum Say, S. vittatum Zett., S. corbis n. sp. and S. nigroparvum n. sp. found here.*

*Simulium (Simulium) ottawaense* N. SP.

*Female.*—Length, 2–3 mm.; wing, 2.8–3.5 mm. Very close to *venustum* and rather difficult to distinguish from it: somewhat larger and more robust than my specimens of *venustum;* front opaque grayish pollinose; the pale hairs on stem vein intermixed with a few dark hairs (one specimen with these hairs largely dark); terminal abdominal segments grayish-black, sub-opaque. *Genitalia* (Fig. 15, A, 4, 5): ovipositor valves conical, about as long as wide at base; weakly chitinized on inner margins; evenly setose. Anal lobe dorsally about one-third length of cercus, expanding to twice its length ventrally, broadly and roundly produced; bare on anterior margin, a few spicules below; elsewhere more or less evenly setose. Cercus rounded quadrate, a little wider than long; setose. Arms of genital rod moderately divaricate, angulate, outward expansions each with lower margin extended into a broad, long, roundly-pointed tooth.

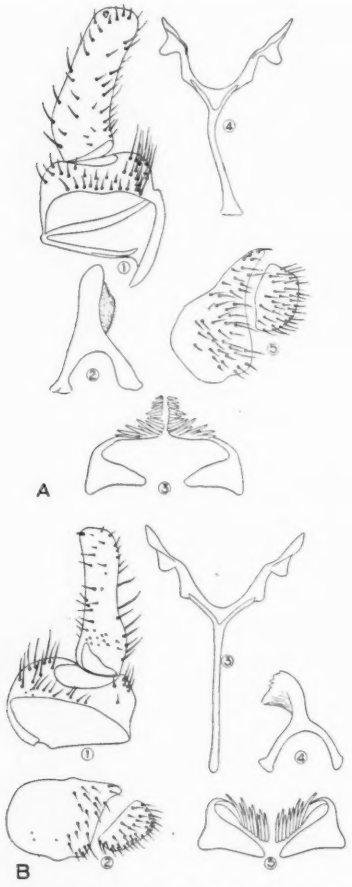


FIG. 15. *A. Parts of genitalia of Simulium (Simulium) ottawaense n. sp.: male (1) side-piece and clasper, (2) adminiculum, (3) adminiculum arms; female (4) genital rod, (5) anal lobe and cercus. B. Parts of genitalia of Simulium (Simulium) corbis n. sp.: male (1) side-piece and clasper, (4) adminiculum, (5) adminiculum arms; female (2) anal lobe and cercus, (3) genital rod.*

*Male.*—Very similar to the male of *venustum*, but general appearance somewhat paler, the white pollinosity on the thorax more opaque, the legs less heavily chitinized (observable in cleared specimens.) *Genitalia* (Fig. 15, A, 1–3): side-piece rounded quadrate, nearly as long as wide, reinforced with a transverse bar of denser chitin as figured. Clasper broad, somewhat strap-shaped, a little more than one and one-half times as long as side-piece; a spine at tip. Adminiculum inverted Y-shaped, strongly chitinized; narrow, laterally compressed, with a rounded, setulose, posterior process or keel; basal arms stout, oblique, truncated. Adminiculum arms held on a convex membrane; chitinized, slender medially, and with a row of rather numerous, close-set short to long teeth; broad laterally.

*Pupa.*—Length about 4 mm. The respiratory tuft consists of eight fila-

ments, and is slightly more than one-half the length of the pupa. The respiratory filaments arise from a short main trunk: six are in pairs, one upper pair and two lower; two are unpaired. One of the unpaired filaments arises directly from the apex of the main trunk, the other from near the base of the upper stalk. The stalks bearing the two lower pairs of filaments are unequal in length, the upper being about one-half that of the lower. The cocoon is of the wall-pocket type; loosely woven, especially anteriorly, the anterior border not thickened to form a rim.

*Holotype.*—Male, Pinks lake, near Hull, Que., 26. VII. 35 (reared from pupa). Can. Nat. Coll. (slide).

*Allotype.*—Female, Carleton Place, Ont., 7. VIII. 35; other data as above.

*Paratypes.*—Seven females, five males (reared from pupae).

*Notes.*—The type material was reared from pupae collected in the Leamy creek close to its source, at Pinks lake, in the Gatineau hills, near Hull, Que., and in the Mississippi river, at Carleton Place, Ontario. In the Mississippi river (Fig. 9, B) several pupae, and numerous larvae, were found (Aug. 5) with *vittatum* on pieces of *Myriophyllum* entrapped in the fast-flowing water below a concrete dam in the town. The temperature of the water was 73° F. Adults emerged, August 6–9. The Leamy creek, at Pinks lake, is a tiny stream flowing down a wooded hillside. The gradient is slight close to the lake where the collection was made, and the flow was only about one foot per second over the stone and debris-strewn bottom. The water temperature was 76° F. (July 24). Scattered pupae and pupal skins of *vitattum* and an occasional pupa of *aureum* were also present. Adults emerged on July 25–26. I have collected pupae of this species in late May and June from several streams in the Ottawa district, during recent years, but had not previously reared the adults. I also found them in the Madawaska river, Algonquin Park, Ontario, on June 30, 1931. The larvae and pupae usually occur in mixed infestations with *venustum* and *vittatum*.

*Simulium (Simulium) corbis* N. SP.

*Female.*—Length about 2.2 mm.; wing, 3.4 mm. Close to *venustum*, and very similar in size, appearance and external characters, but differing as follows: antennae almost entirely black, the basal joints only faintly reddish; pubescence on front, vertex and mesonotum yellow, somewhat more scale-like; upright marginal hairs on scutellum pale yellow and black; hind basitarsus about 5 × 1; claws with a small but distinct, pointed, sub-basal tooth. *Genitalia* (Fig. 15, B, 2, 3): ovipositor valves about as long as wide, tips rounded; largely membranous, slightly embrowned on inner margins; minutely setulose; each with a median group of spicules. Anal lobe quadrate, rounded anteriorly; as long as ninth tergite, and one and one-half times as wide as cercus (vertical width); minutely setulose; posterior third moderately setose; a group of tiny spicules antero-ventrally; dorsal and ventral margins each with a small,

minutely spiculate, rounded protuberance. Cercus rounded quadrate, wider than long; setose. Arms of genital rod angled on lower margins; outward expanded portion more strongly chitinized, and with a broad, blunt tooth.

*Male.*—Similar in all respects to the male of *venustum* except as follows: pleural membrane reddish, tarsal joints of all legs entirely black; hind basitarsus about 4 × 1 and four-fifths width of tibia. *Genitalia* (Fig. 15, B, 1, 4, 5): side-piece and clasper similar to *ottawaense*; side-piece rounded quadrate, about as long as broad, a reinforcing chitinous bar present, but less distinct; claspers about one and one-half times as long as side-piece, broad, constricted slightly at middle, the setae on undersurface unusually long and stout; a slender spine at tip. Adminiculum narrow, strongly chitinized, inverted Y-shaped viewed dorsally; produced posteriorly into a flat, apically rounded process or keel, sparsely and minutely setulose on the sides, and marginally denticulate, the denticles backwardly directed, sharply pointed, sub-equal; basal arms stout, oblique, truncated. Adminiculum arms on a supporting convex membrane, chitinized, and with a row of rather long teeth; the arms broadening and triangulate laterally.

*Holotype.*—Female, Blanche river, near Perkins Mills, Que. (reared). Can. Nat. Coll.

*Allotype.*—Male, same data.

*Paratypes.*—Ten females, four males, same data.

*Pupa.*—Length about 3.5 mm.; respiratory tuft approximately three-eighths as long as the pupa. Each tuft consists of ten filaments of about equal length and thickness, arising in sessile pairs from a short, common base, and held fanwise, all more or less in the vertical plane. The cocoon is slipper-shaped, about 5 mm. long; the posterior two-thirds rather closely woven: anterior to this portion, on each side, is an elongate opening next to the anterior border; extending beyond the border, except narrowly dorsally, and forming the cuff of the slipper is an interlacing network of filaments, like the ornamental rim of a basket. The pupa lies in the cocoon with the respiratory tufts protected by this basket-work extension, through the meshes of which the head and respiratory filaments are visible.

*Notes.*—The pupae were found on May 22, 1935, on the submerged stems of dogwood growing close to the bank of the Blanche river, in the rapids, a short distance below a waterfall, five miles south of Perkins Mills, Que. (Fig. 7, A). The river was flowing swiftly over a stony bottom; the temperature of the water was 53° F. Pupae of *venustum* and *quebecense* occurred on stones nearby. Eighteen males and twelve females of *corbis* had emerged by May 26; several were damaged and preserved in alcohol-glycerine, the others pinned.

## Acknowledgments

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# *TETRAMERES CRAMI* SWALES, 1933, A NEMATODE PARASITE OF DUCKS IN CANADA. MORPHOLOGICAL AND BIOLOGICAL STUDIES[1]

By W. E. Swales[2]


## Abstract

The male of *Tetrameres crami* is described and the female is redescribed in the light of further studies. The life history has been elucidated and the larval stages described. Two fresh-water amphipods, *Gammarus fasciatus* Say and *Hyalella knickerbockeri* (Bate) are intermediate hosts. A list of definitive hosts, notes on the ecology of the intermediate hosts and on tetrameriasis crami, are included.


The original description of *Tetrameres crami* was based on the morphology of nine female specimens collected from a domestic duck from Ottawa, Ontario. Since that time this nematode has been found to be the most common and probably the most important helminth parasite of domestic ducks in the province of Quebec. It has also been found as a parasite of 12 species of wild ducks from both eastern and mid-western Canada. Morphological studies of a large amount of material have shown that the original description of the female must be considerably modified, and this redescription, together with a description of the male, has been included herewith.

The studies on the life history, which involves an amphipod as intermediate host, indicate that this nematode is probably widespread in North America.

## Family Tetrameridae Travassos, 1914

*Morphology*

*Tetrameres*. Female body globular or spindle-shaped, with four longitudinal furrows corresponding to the median and lateral lines. Mouth of male generally with two lateral trilobed pseudolabia and a dorsal and a ventral interlabium; female lips indistinct or lacking. Buccal capsule with chitinoid walls. A slender muscular oesophagus surrounded by the nerve ring and followed by a more robust glandular oesophagus.

### *Tetrameres crami* Swales, 1933

*Male.*—Filiform, 2.9–4.1 mm. long by 70–92$\mu$ wide. Cuticle adorned with two double rows of spine-like sensory organs which extend from the anterior end to the cloacal region. The "lips" conform to the description of those of *T. americana* and *T. fissispina* by Chitwood and Wehr (1), there being two lateral trilobed pseudolabia and two inconspicuous interlabia. Two papillae are situated at the base of each interlabium, one on each side; one smaller papilla is situated in the middle of each pseudolabium, slightly above the others. The cleft or spear-shaped cervical spines extend from the

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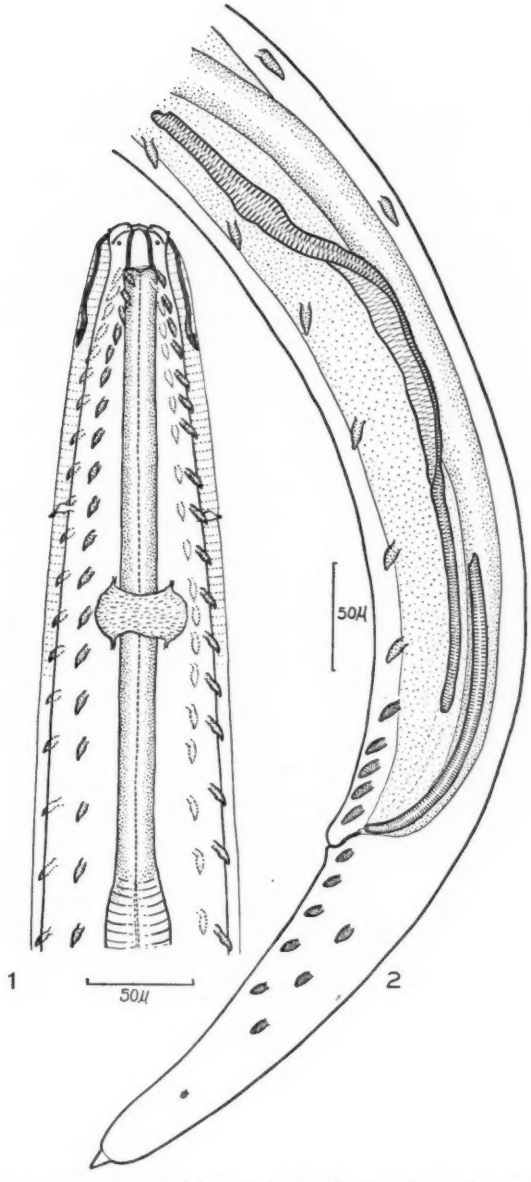
anterior part of the head as modified lateral alae; they are 60$\mu$ in length. Buccal capsule roughly bell-shaped and strongly chitinoid, 15–20 $\mu$. long by 9–11$\mu$ wide. Muscular oesophagus (11) 0.281–0.325 mm. in length, surrounded near its middle by a prominent nerve ring. Glandular oesophagus 0.730–0.875 mm. long by 42–56$\mu$ in maximum width. The cervical papillae, distinct from the spine-like sensory organs, may be equidistant from the anterior end of the body or one slightly anterior to the other; the ranges of distances are 0.116 mm. and 0.122 mm. to 0.145 mm. The tail, of more or less constant length in most nematodes, is rather variable; distances from the cloacal aperture to the tip range between 0.148 mm. and 0.248 mm., the commonest distance being 0.195 mm. Specimens from wild ducks frequently showed a greater tail length than those from domestic ducks but this was later shown to be due to the fact that the former birds were seldom obtained in a fresh state and that slight decomposition caused minor deformities in the nematodes. The tip of the tail is in the form of a cuticular cone, 10$\mu$ in length.

Spicules unequal, not strongly chitinoid; the right one narrow and curved, 0.136–0.185 mm. in length. The left spicule larger and twisted, 0.272–0.350 mm. in length. Testis about 2.0 mm. long, reflexed at a point about 0.150 mm. posterior to the oesophagus; vas deferens robust, about 0.8 mm. long, the junction with the testis being conspicuously constricted.

The cuticular processes, the "spines" of other described species, appear to be all sensory organs, there being a process from each "spine" which passes through the cuticle into the hypodermis; all the tips are refractive, and increasingly so towards the posterior end of the body. With the advice of Dr. B. G. Chitwood, who kindly examined some of my specimens, I have tentatively differentiated the posterior 14 pairs as genital papillae, on account of their position, the larger refractive tips and decreased length. These papillae are distributed as five pairs preanal and sub-ventral, one pair adanal, four pairs postanal and sub-ventral, and four pairs in a lateral position on the tail. The posterior pair of the "lateral" four pairs are small and inconspicuous. The adanal papillae are postanal in some specimens, thus giving these individuals five pairs of postanal papillae in the sub-ventral rows. The somatic sensory organs are somewhat longer, have smaller refractive tips, and number from 49–55 in each row; they range from 10$\mu$ long at the anterior end of the body to 18$\mu$ long in the region just anterior to the spicules. Postanal genital papillae 8–9$\mu$ in length, with the exception of the last pair which are much smaller.

*Female.*—Globular to spindle-shaped, 1.5–3.25 mm. in length by 1.2–2.2 mm. in maximum width. Live specimens blood-red in color and able to change their shape very considerably by waves of contraction and expansion. The anterior end protrudes from the globular body for a distance of 0.84 mm. in fully grown specimens, and the protruding posterior part is 0.5 mm. long. Cervical papillae approximately 0.214 and 0.241 mm. from the anterior extremity.




FIGS. 1 AND 2. 1. *T. crami, adult male, head end.* 2. *Adult male, caudal end. The genital papillae shaded more heavily than the somatic sensory organs.*

Buccal capsule or head capsule sub-circular to barrel-shaped, 12–14$\mu$ long by 11–12.2$\mu$ wide. Muscular oesophagus 0.22–0.247 mm. long, surrounded just posterior to its middle by the nerve ring; glandular oesophagus 0.916–1.3 mm. long. Intestine saccular, about 2.0 mm. long by 1.0 mm. wide in fully grown specimens. Anus 0.113–0.156 mm. from the tip of the tail. The tail ends in a small cuticular cone.

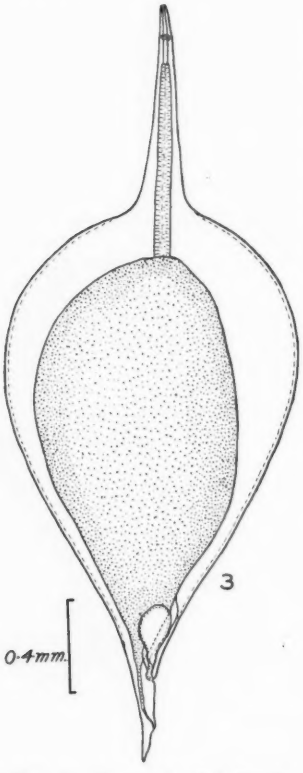



FIG. 3. *T. crami, adult female.*

Vulva situated in a deep depression in the posterior projection near the body proper, 0.319–0.350 mm. from the caudal extremity. The short vestibule of the ovejector is joined on its ventral side by a copulatory receptaculum at a point 50$\mu$ from the vulva. Receptaculum thick-walled and saccular, 0.3 mm. long by 0.15 mm. wide. The trompe bifurcates at a point ventral to the intestine and 0.5–0.6 from the vulva, and the branches join the uteri, which together with the long filiform ovaries, fill the body cavity. Eggs oval and fully embryonated when laid; they are 41–57$\mu$ long by 26–34$\mu$ wide, the commonest size of 200 specimens measured being 53$\mu$ long by 28$\mu$ wide.

*Primary Hosts:*

*Anas boschas domesticus,*
*Anas platyrhynchos,*
*Anas rubripes,*
*Chaulelasmus streperus*
*Mareca americana,*
*Nettion carolinense,*
*Querquedula discors,*
*Spatula clypeata,*
*Dafila acuta,*
*Nyroca marila,*
*Nyroca affinis,* and
*Glaucionetta clangula.*

*Intermediate Hosts:*

*Gammarus fasciatus* Say and *Hyalella knickerbockeri* Bate.

*Location:*

Proventriculus (glandular stomach).

*Distribution:*

Canada (Provinces of Quebec, Ontario, Manitoba, Saskatchewan and Alberta) and probably throughout North America.

## The Life Cycle of *T. crami*

The search for the intermediate hosts of this nematode was confined to aquatic arthropoda owing to the fact that the adult parasite was only found in ducks of the Subfamilies Anatinae and Fuligulinae. Because of the prevalence of *Daphnia pulex* and because this crustacean had been reported as an intermediate host of *Tetrameres fissispina*, large numbers of this species were exposed to feces of infested birds and to heavy suspensions of embryonated ova. *D. pulex* ingests the ova readily but in no case have the ova been found to hatch, but are passed out unchanged. Examination of approximately 200 specimens which had been suitably exposed some days previously failed to reveal the presence of larvae. Other Entomostraca tested and found negative are *Daphnia longispina*, *D. magna*, *Daphnia* sp., *Ceriodaphnia* sp. and several species of Cladocera.

Following the tests of the Entomostraca a search for Amphipoda revealed the presence of two common species in this district, *Hyalella knickerbockeri* (Bate) and *Gammarus fasciatus* Say. Two specimens of the former species were exposed to a suspension of ova obtained by dissection of female nematodes. Seven days later these amphipods were found to have several active larvae in their body cavities.

A number of specimens of *Gammarus fasciatus* was similarly exposed and subsequent examination revealed that all the specimens contained larvae, some being very heavily infested. After five or six days the larvae can be readily seen in most specimens of both of the above-mentioned amphipods by examining the live animal under a dissecting microscope. Data concerning the development of the parasite in the intermediate hosts were obtained from a series of experimental infestations of both intermediaries.

The ova, which are infective when laid, are readily ingested by the amphipods in water. Ova stored in water at 4–5° C. for ten months were found to be still viable, and were used for experimental infestations. They hatch in the digestive tract and migrate to the body cavity. In 12 to 18 hours the larvae are easily found by dissection of the amphipods; measurements of these first stage larvae have shown them to be 0.197 mm. to 0.223 mm. in length by 12$\mu$ to 13$\mu$ in width. At this stage the larvae are very inactive and rapidly die and disintegrate in water. They are comparatively thick, their anterior ends being rounded and transversely striated; details of morphology are difficult to determine, but the head end is blunt, with no lips, and the tail end is bluntly rounded.

In from seven to eleven days after exposure second stage larvae are seen in the body cavity. These forms are from 0.279 mm. to 0.95 mm. in length by 39$\mu$ in maximum width and can be distinguished by the rounded caudal extremity; under the cuticle of this extremity can be seen the caudal papillae of the third stage (Fig. 4). The size to which this stage can grow before it moults appears to be very variable, as third stage larvae slightly more than 0.45 mm. in length and 33$\mu$ in width have been found. The second stage

larvae, after the period of activity and growth, migrate to various parts of the body and become encysted. Common locations of the cysts are the coxal plates and on the inner surface of the dorsal shield; in some cases they have been observed among the muscles of the peraeopods, apparently interfering with normal muscular activity. The cysts are in the form of thin-walled and egg-shaped formations loosely attached to the inner surfaces of the chitinoid structures of the amphipod. They are approximately 0.33 mm. by 0.27 mm. in size; they are easily ruptured by slight pressure of a cover-slip. The encysted larvae assume various coiled or "figure-eight" positions, which they frequently change.

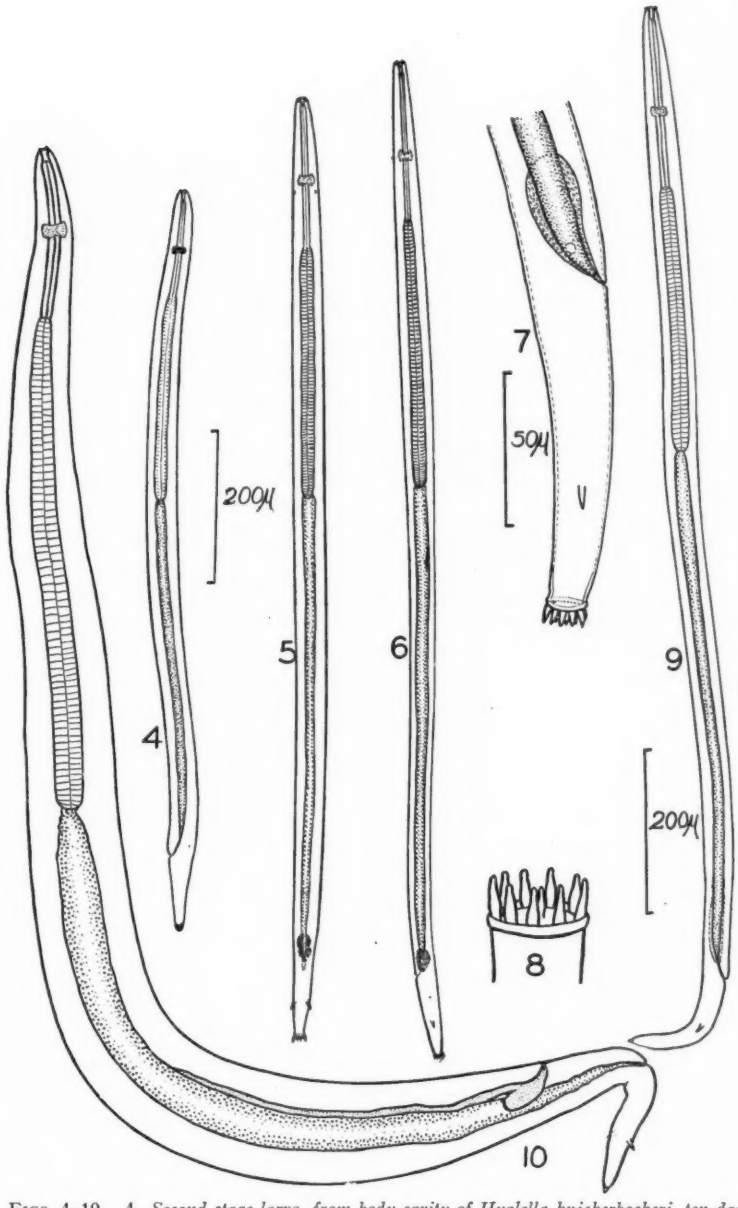
Although it is the rule for the second stage larvae to encyst, instances have occurred in experimentally infested amphipods of a moult to the third stage prior to encystment. Usually, however, the moult occurs within the cyst, and development continues in this position.

The third stage encysted larvae have been found to vary greatly in size, 0.455–1.406 mm. in length by 33–45$\mu$ in width. These measurements were recorded from specimens encysted in *Hyalella knickerbockeri* for from 20 to 65 days, but the largest larva (1.406 mm. long) developed in a second generation *H. knickerbockeri* in 24 days. This rapid growth of the encysted larva has not been found to involve another moult and each specimen had the general structural characteristics which appear to be constant within the stage. The anterior extremity has four oral papillae; the cervical papillae are approximately 0.130 mm. from the anterior extremity. Buccal capsule cylindrical, 11–11.8$\mu$ long by 5–6$\mu$ wide. Muscular oesophagus 0.152–0.211 mm. long; glandular oesophagus 0.268–0.390 mm. long by 25$\mu$ in maximum width. Tail 0.097–0.120 mm. long, gradually tapering to a wide tip. Situated laterally on the tail 50$\mu$ from the tip is a pair of spine-like sensory organs about 14$\mu$ long, similar in outline to those on the body of the adult male, and having a fine basal process and a refractive tip. The end of the tail is cylindrical, and is about 13$\mu$ in diameter; it is adorned with a circle of nine papillae situated on the inner edge of a cuticular ring, and one papilla is situated on the tail end in the centre of the ring (Fig. 7). The outside papillae are of equal length (6–8$\mu$), the central one being somewhat smaller.

The genital primordium is readily seen in this stage as a small mass near the end of the intestine.

## Development in the Final Host

Experiments involving infestation of domestic ducks and "tame" mallards *(Anas platyrhynchos)* were conducted to demonstrate the infectivity of the third larval stage and to complete cross infection experiments from wild to domestic ducks and *vice versa*. The life cycle was experimentally completed from *Anas rubripes* through *G. fasciatus* to *Anas platyrhynchos*, from *A. rubripes* through *G. fasciatus* to *Anas boschas domesticus*, from *A. boschas domesticus* through *G. fasciatus* to *A. boschas domesticus* and from *A. boschas domes-*




FIGS. 4–10. 4. *Second stage larva, from body cavity of Hyalella knickerbockeri, ten days after ingestion of ova.* 5. *Third stage larva, from Gammarus fasciatus, 32 days after infestation. (Same scale as Fig. 4.)* 6. *Third stage larva from the proventriculus of a naturally infested domestic duck. (Same scale as Figs. 4 and 5.)* 7. *The caudal end of a third stage larva from H. knickerbockeri, 60 days after infestation.* 8. *A diagrammatic illustration of the papi.lae on the end of the tail of a third stage larva.* 9. *Fourth stage larval male from the proventriculus of a naturally infested duck.* 10. *Fourth stage larval female. (Same scale as Fig. 9.)*

*ticus* through *H. knickerbockeri* to *Anas platyryhnchos*. The domestic ducks and the mallards used in the experiments had been hatched in an incubator and raised in this laboratory. Details of the experiments are as follows:—

*Experiment I*

Ova obtained by dissecting female tetrameres from the proventriculus of *Anas rubripes*, were placed in a pint jar of water containing a number of *G. fasciatus*. Five days later (November 4, 1935), the amphipods were removed to a jar of clean water, examination of four having revealed the presence of larvae in their body cavities. On November 18, 1935, six specimens were examined and found to contain encysted larvae; these amphipods were placed in a gelatine capsule and fed to a domestic duck. No nematodes were found on autopsy on December 14, 1935.

*Experiment II*

Five *G. fasciatus*, from the same lot as those in Experiment I. which were seen to contain encysted larvae, were fed to a domestic duck on November 24, 1935. Autopsy on December 27, 1935, did not reveal any nematodes in the proventriculus.

*Experiment III*

Ova from nematodes in *A. rubripes* were fed to *G. fasciatus* on November 7, 1935. The amphipods were removed to clean water 24 hours later. On December 7, 1935, two of these amphipods, one apparently containing one encysted larva and the other ten, were fed to a seven-month-old mallard. This bird was killed and autopsied on January 7, and eight female tetrameres ranging in size from 1.03 mm. by 0.578 mm. to 2.48 mm. by 1.07 mm. were found in the crypts of Lieberkühn. In the mucous lining were found one immature male, 1.5 mm. long by 47$\mu$ wide, and one-third stage larva. The females did not contain embryonated ova but in other respects were essentially the same as those obtained from natural infestations.

*Experiment IV*

Two *G. fasciatus* from the same lot as those in Experiment III were seen to contain several encysted larvae. These amphipods were fed to a domestic duck on December 12, 1935 (33 days after ingestion of ova). The duck was killed and examined on February 11, 1936, and one adult female tetramere and an immature male were recovered from the proventriculus.

*Experiment V*

Numerous specimens of *H. knickerbockeri* were exposed to ova freshly dissected from females obtained from *Anas rubripes* on November 5, 1935. Ten of these amphipods, each apparently parasitized by one encysted larva, were fed to a domestic duck on December 15, 1936. The autopsy on January 11, 1936 did not reveal any helminths.

*Experiment VI*

On November 8, 1935, several *G. fasciatus* were exposed to ova obtained from female tetrameres from a naturally infested domestic duck. Three of these amphipods, each apparently containing one encysted larva, were fed to a domestic duck on December 15, 1935. The autopsy on January 11, 1936, revealed one sexually immature female tetramere, 1.7 mm. long by 1.0 mm. wide.

*Experiment VII*

On November 8, 1935, numerous specimens of *H. knickerbockeri* were exposed to ova from tetrameres from a naturally infested domestic duck. Five of these amphipods were fed to a mallard on January 13, 1936. This bird was killed on February 15, 1936 and one mature male tetramere, three mature females and one immature female tetramere were recovered. The male was 3.4 mm. long by 89$\mu$ wide, tail 0.193 mm. long, spicules 0.330 mm. and 0.162 mm. long. The mature females were 1.5 mm. by 1.0 mm., 2.2 mm. by 1.5 mm. and 2.0 mm. by 2.0 mm. in size.

*Experiment VIII*

Ten infested specimens of *H. knickerbockeri* from the same lot as those in Experiment VII were fed to a two-month-old chicken in June, 1936. The autopsy ten days later failed to reveal any tetrameres.

In the above experiments all the ducks used were free of helminths other than *T. crami*. Owing to the fact that they had never had access to water other than the filtered laboratory supply, controls were dispensed with. The lack of uniformity of development and the failure of the duck in Experiment V to become parasitized appears to indicate individual resistance.

The experiments indicate that the third stage larvae require some time for growth in their encysted state before they are infective for ducks. They can be infective after 29 days in the intermediate hosts and it is probable that infectivity may be attained in a somewhat shorter period. The sexually mature adult state can be reached in the proventriculus of ducks in 33 days after ingestion of infective larvae. However, owing to the previously noted irregularity in growth-rate in the experimentally infested ducks, it seems inadvisable at this time to state definite periods necessary for development.

The fourth stage larva does not conform to the description of that of *T. fissispina* by Seurat (8). I have determined this stage to be the form found in the crypts of Lieberkühn in which the character of the tail-ending has been changed from the ring of papillae to a cone-shaped organ similar to that of the adult; it does, however, retain the lateral sensory spines. This stage exhibits marked sexual differentiation, the male is similar in outline to the adult male, and the female body is wide and the enlarged intestine usually contains some ingesta. The fourth stage larval male is 1.36–1.41 mm. in length by 36–48$\mu$ wide; the buccal capsule roughly cylindrical, 13–14$\mu$ long; muscular oesophagus 0.17–0.2 mm. long, glandular oesophagus 0.330–0.386

mm. in length. Tail 0.13–0.15 mm. long, ending in a cuticular cone 8–9$\mu$ in length. Sensory spines of the tail 0.08–0.1 mm. from the caudal tip. The outlines of the genital structures were not clear in the available specimens.

The fourth stage larval female is dark in color owing to the intestinal mass. The available specimens measured 1.7–1.93 mm. long by 0.120–0.132 mm. wide. Buccal capsule cylindrical, 14$\mu$ long and somewhat wider than that of the male. Muscular oesophagus 0.2–0.23 mm. long; glandular oesophagus 0.53–0.6 mm. long. Tail 0.12–0.13 mm. long, ending in a cuticular cone 8–9$\mu$ long. The sensory spines approximately 70$\mu$ from the caudal tip. A single outstretched genital tube, up to 0.46 mm. long, joins a small undeveloped copulatory receptaculum near the rudimentary vulva which is covered by the larval cuticle.

In considering the forms described above as the fourth larval stage, several points worthy of note were taken into consideration. The form described as the third stage and infective larva grows considerably in size and has been found to live for a considerable time in both the intermediate and definitive hosts. Although many specimens have been examined no evidence of a moult to another similar stage has been observed. The general structure of all these specimens has been the same, but each organ has shown evidence of growth. By analogy, it would appear that the form described by Seurat (8) was the third larval stage of *T. fissispina*. The stage described above as the fourth, however, does exhibit characteristics that indicate a necessary moult to the adult state although, except in the region of the vulva of the female, the larval cuticle is not well marked. The sensory spines are probably the best criteria of this stage, as it is unlikely that these organs could be cast off without a complete ecdysis. Unfortunately I have been unable to find a specimen in the act of its final moult.

Only adult males and third stage larvae have been found in the mucus of the proventriculus; thus it is apparent that the fourth larval stage exists entirely in the crypts and that only the adult male returns to the surface.

The immature adult female quickly assumes the globular shape and the specimens observed are slightly tinged with red and considerably shorter than the fourth stage larvae. The longitudinal furrows are well marked. Sensory organs on the tail are lacking.

### *T. crami* Compared with Other Known Species of the Genus

A consideration of the description of the adult male, the modified morphology of the female and the studies on the life history reveal that *Tetrameres crami* is more closely related to *T. fissispina* than to any of the other known species. The descriptions of the latter species by various authors are somewhat contradictory. According to Wharton (*vide* Cram (2) and Hsü (7)), *T. fissispina* is a much larger species; according to Travassos (14), this species is of similar size but differs considerably from *T. crami* in the length of the female tail, the sizes of the buccal capsules, the length of the male oesophagus

and the spicule lengths, as well as in other minor points. *T. crami* has not been found as a parasite of *Gallus gallus*, which is a common host of *T. fissispina*. All attempts to infest *Daphnia pulex*, a reported intermediate host of *T. fissispina*, with *T. crami* have failed.

The other known North American species of *Tetrameres*, *T. americana* Cram, 1927 and *T. pattersoni* Cram, 1933, cannot be confused with *T. crami*. Both are very much larger species, and have gallinaceous birds as definitive hosts and Orthoptera (grasshoppers and cockroaches) as intermediaries.

*T. paucispina* Sandground, 1928, from a South American "black-bird", differs chiefly in the length of the male tail. All other species have distinct structural differences as well as different hosts. *T. fissispina*, *T. americana*, *T. pattersoni* and *T. crami* are the only species of the genus for which the life cycles are known.

## Tetrameriasis Crami

The effect of *T. crami* upon the definitive host has not yet been fully determined, but a study of the histopathology of tetrameriasis crami is being continued.

In infestations involving the presence of one hundred or more female nematodes in the crypts of Lieberkühn the host has always been found in rather poor condition. In the few cases studied from this aspect, sections of the crypts have revealed the adult parasites to be enclosed in a thin fibrous cyst around which little or no evidence of acute inflammatory processes can be seen. However, the crypt is greatly distended and, owing to mechanical blockage, the secretory glands cannot function. As the function of the proventriculus is to bathe the passing food with gastric juice and pepsin, and thus break down protein, severe infestations probably impair this process to some extent. This effect alone may account for the failure of the parasitized birds to gain weight normally.

The females of this genus are known to feed on blood, this fact having been substantiated in the case of *T. crami* by examinations of intestinal detritus. The intake of blood for food requirements must be fairly extensive in the case of an adult egg-producing female. The toxic action decribed in tetrameriasis fissispina of pigeons by Timon-David (12) may also be found to play a part in this disease. Cram (3) notes that in tetrameriasis americana of *Gallus gallus* there is a catarrhal condition associated with a thickening of the walls of the proventriculus. This condition has been noted in a few cases in this study, but it is not constant in heavy infestations with *T. crami*.

The alternate orientation of the females in the crypts in heavy infestations of *T. fissispina* of pigeons as described by Timon-David (12), has not been observed in the case of *T. crami*.

## Notes on the Intermediate Hosts

*Gammarus fasciatus* and *Hyalella knickerbockeri* have a wide range of distribution in North America, and are rather abundant in many bodies of water.

*Gammarus fasciatus* is found commonly on sandy shores of rivers and lakes. In this district of the Province of Quebec it is easily collected in the late

summer and autumn by sweeping a sieve through aquatic vegetation in from 12 to 18 inches of water. The most favorable habitat observed in 1935 was a favorite feeding ground of wild ducks; this habitat is a shallow sandy bay on Lake St. Louis, containing an abundance of aquatic vegetation chiefly represented by *Vallisneria americana, Elodea canadensis, Potamogeton Richardsonii, Potamogeton filiformis* and *Chara* spp. A few days prior to the freezing over of the shallow water, masses of these plants were washed up near the shore, and large numbers of *G. fasciatus* were collected from this vegetation. Even after most of the water along the shore was frozen over, small open holes yielded hundreds of these amphipods. These animals were kept alive for some weeks in the laboratory in glass tanks provided with sand, clear water and *Vallisneria americana*.

*G. fasciatus* becomes infested with *T. crami* very readily but the second stage larvae appear to exert a marked pathogenic effect upon this amphipod. In heavy infestations the animal becomes yellow in color and so feeble that it may be imprisoned by the surface film. Many of my specimens which contained third stage infective larvae exhibited the same symptoms of weakness and this would make them easy prey for the final host under natural conditions.

*Hyalella knickerbockeri* is usually found in an entirely different habitat from that mentioned above. It is a common inhabitant of ditches, creeks, quiet muddy bays of lakes, small rivers and ponds. Quantities of algae appear to be essential for its optimum condition of life, although dead leaves of trees may also serve as food. A handful of filamentous algae from a muddy shore of a creek will usually yield many specimens of this amphipod. It is very easily kept alive in the laboratory, and second generation specimens are readily obtained if the adults are placed in a small jar with mud and algae. Changes of water are undesirable and aeration is unnecessary. Specimens have been kept alive in a pint jar for over ten months and are still living, water having been added monthly to replace that lost by evaporation.

*H. knickerbockeri* is easily infested experimentally with *T. crami*. They do not become so heavily infested as *G. fasciatus* however, even under identical conditions of exposure; the largest number of larvae found in a single specimen was four. This amphipod does not appear to be affected to any visible extent by the infestation; infested specimens have been kept in aquaria for over five months and on being dissected at the end of this time yielded active larvae.

Of the above-mentioned intermediate hosts, *G. fasciatus* appears to be the one chiefly responsible for the infestations in wild ducks, owing to the fact that it is the species commonly present on the typical feeding grounds of scaups and other diving ducks. On the other hand, *H. knickerbockeri* is probably responsible for the infestation of domestic ducks because these birds do not usually have access to sandy shores of large lakes but they frequently feed in ponds, creeks, etc.

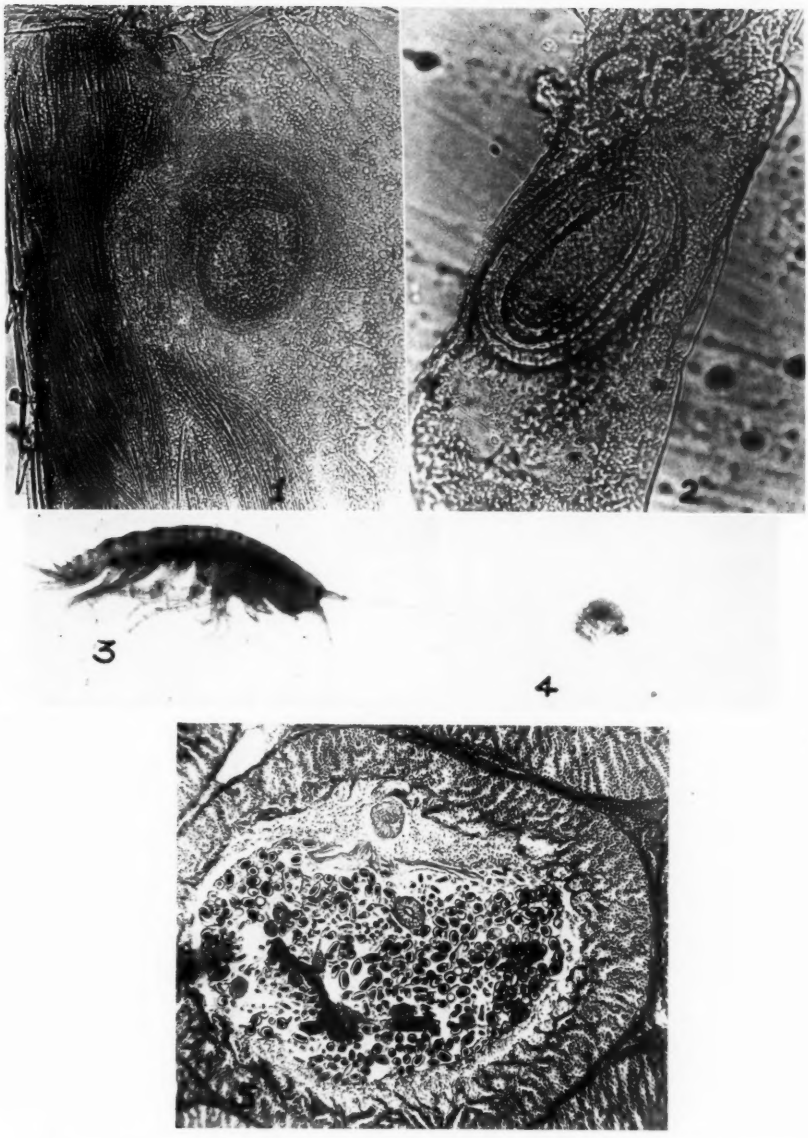



**Plate I.** *A third stage larva encysted in a peraeopod of Gammarus fasciatus. (×110). 2. A third stage larva encysted on a thoracic somite of Hyalella knickerbockeri. (×143). 3. Gammarus fasciatus* Say. *(×5). 4. Hyalella knickerbockeri (Bate). (×3·5). 5. Section of a female of T. crami in a crypt of the proventriculus of a duck.*

Both *G. fasciatus* and *H. knickerbockeri* form an important part of the food of many fishes. A single adult stickleback *(Eucalia inconstans)* placed in a tank containing *H. knickerbockeri* was found to have ingested ten of these amphipods in one hour. The fact that so many of our waters have been badly depleted of fish may explain the prevalence of the amphipods and hence the prevalence of *Tetrameres crami*. Readjustment of the aquatic fauna in areas where tetrameriasis crami is found to be of economic importance should tend to prevent harmful infestations of ducks.

Attempts to destroy amphipods by chemical means have not shown any promise. For example, a 1% solution of copper sulphate killed all the amphipods in one hour, but a 1 : 500,000 solution failed to kill a mixed lot in 24 hours; these tests were conducted in jars free from organic matter.

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